AN ABSTRACT OF THE THESIS OF

Karla Gail Lamka for the degree of Master of Science

in Microbiology presented on August 29, 1979

Title: Specific Indicator Organisms Can Define the Magnitude and Origins of Non-point Pollution in Rural Environments

Abstract approved: Redacted for Privacy

Ramon J. Seidler

A comprehensive study was made on the impact to surface and ground water pollution by a recently populated rural neighborhood located in the foothills of western Oregon. A stream originating in a limited access forest and flowing through a modern zoned housing development was routinely monitored. Sampling was performed during steady state low flow and high flow conditions, and during the rising and falling hydrograph of storm events. Bacterial levels were found to be one to two log units higher in the unprotected regions of the stream compared to levels in the protected forest.

To determine the source of fecal pollution in the stream, fecal streptococci were randomly picked and biotyped. It was demonstrated that sources of fecal contamination in the stream included human feces, as evidenced by the presence of S. mitis and S. salivarius during stormwater runoff. This was attributed to use of septic drain-fields in soils unsuitable for such use. Other contributors were domestic

livestock and wildlife, as manifested by the isolation of S. bovis and S. equinus.

To determine the effects of seasonal variation and modern zoning criteria on drinking water supplies, six surveys over a fifteen month period were conducted. More than one third of the water supplies were unsatisfactory on at least one occasion in terms of standard plate counts over 10^3/ml, the presence of coliforms, fecal coliforms, and/or Staphylococcus aureus. Coliform contamination was found to be higher following periods of rainfall, while high standard plate counts were more prevalent during warmer weather. Consumer education and a twice yearly monitoring of private water supplies appear necessary to reduce the incidence of water-borne disease organisms in private water supplies.

Specific Indicator Organisms Can Define the Magnitude and Origins of Non-point Pollution in Rural Environments

by

Karla Gail Lamka

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed August 29, 1979

Commencement June, 1980

APPROVED:

Redacted for Privacy

Associate Professor of Microbiology in charge of major

Redacted for Privacy

Chairman of Department of Microbiology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented August 29, 1979

Typed by Connie Zook for Karla Gail Lamka

To John Fulton,

for his invaluable support,

encouragement, and love

Acknowledgments

I would like to express my sincere appreciation to:

Dr. Ramon Seidler, for his guidance and support during my time at Oregon State University;

The secretarial staff, for all their friendly assistance;

The graduate students and post-doc of Nash 222, for their help, humor, and harrassment.

This work was supported by the Water Resources Research Institute of Oregon State University.

Table of Contents

List of Figures

List of Tables

SPECIFIC INDICATOR ORGANISMS CAN DEFINE THE MAGNITUDE AND ORIGINS OF NON-POINT POLLUTION IN RURAL ENVIRONMENTS

Chapter 1.

Introduction

Streptococci are gram positive cocci which grow in pairs and chains, are oxidase-negative, and do not produce catalase or reduce nitrates. These organisms have fastidious nutritional requirements, and need enriched infusion medium for growth. Carbohydrate fermentation is homofermentative with dextrorotatory lactic acid as the major end product (40). Streptococci were first described by Billroth in 1874, when he observed globular, chain-forming microorganisms in purulent exudates from erysipelas lesions (55). Similar organisms were subsequently isolated from blood in puerperal fever and from the throat in scarlet fever.

It was later found that a number of different kinds of streptococci could be isolated from humans and animals. To classify them, Schottmuller in 1903 used their ability to hemolyze erythrocytes. In 1905, Gordon was the first to apply fermentation tests in a broad and systematic way. However, such tests were confusing when employed without any prior subdivision of the streptococci into groups. Thus, in 1937, Sherman proposed a classification of the streptococci on the basis of temperature limits of growth, along with certain other tolerance tests (67).

The resulting four taxonomic groups include the pyogenic, viridans, lactic, and enterococcal divisions. The pyogenic group does

not grow at 10C or 45C, while the viridans streptococci grow at 45C but not at 10C. The enterococci grow both at 10C and 45C, and constitute the only group which can grow at pH 9.6 and 6.5% NaCl. The lactic group can grow at 10C but not at 45C. The lactic group and the enterococci are the only streptococci which can grow in 0.1% methylene blue milk.

In 1933, Lancefield published her results on the serology of the streptococci. She was able to extract polysaccharide antigens which divided these microorganisms into 15 groups, designated A through O. Through her work, the contemporary taxonomy of the streptococci was reaffirmed and extended to point out new groups or species of which bacteriologists had been previously unaware (39).

Most of the fecal streptococci possess the group D antigen. However, neither S. salivarius nor S. mitis belong to this group, although some S. salivarius strains do possess group K antigens. Streptococcus avium contains both group Q and group D antigens, but the Q antigen, instead of being an integral part of the cell, can be readily lost (59). Table 1 summarizes the arrangement of the fecal streptococci, according to both Sherman's criteria and Lancefield's serological grouping.

Most of the group antigens are composed of rhamnose and glucosamine (56) and are located in the cell wall of these organisms. However, the group D and group N antigens are atypical in being undetectable in cell wall extracts of pure cultures. Instead, they are located beneath the cell wall, at the surface of the protoplast (70). These antigens are also unique in that their chief component is a glucosyl-glycerophosphate.

While the serology of the microorganisms is a valuable taxonomic tool, it does have limitations. In a study performed by Krantz and Dunne, it was found that 42% of 557 streptococci from domestic animals could not be grouped (38). Instead of an absolute correlation between antigenic group and animal species, Lancefield's groups are very widely distributed among animals and pathological conditions.

Lancefield also found that one could "type" species within these groups. Among the group D streptococci, the type antigens, which currently include 39 serotypes, are located within the cell wall. These serological types occupy a wide range of ecological distribution and of fermentation reactions, particularly those types which contain a large number of strains (5). Further research in this area, along with use of fluorescent antibody techniques, has been recommended (24,62).

Another taxonomic tool, bacteriophage typing, was originally considered a stable quality, but Groman and Brock found that phage sensitivity did not correlate with any other characteristic of the group, and that sensitivity "varied in a capricious and unpredictable manner" (9).

Deibel used hemolytic reactions as a helpful quality in differentiating S. faecium from S. durans, and in identifying S. faecalis var. liquefaciens (17). These hemolysins were easily lost, however, and Jacob discovered them to be on a high frequency recombinant plasmid, proving their unreliability (34).

In 1963, Brock et al. studied bacteriocin production by enterococci (11). They characterized these bacteriocins by their action against indicator organisms, and their sensitivity to chloroform, heat, and proteolytic enzymes. Differentiated in this way, there are five major groups of bacteriocins. These types are not linked to specific biotypes; instead, the production and the sensitivity to them appeared randomly distributed among the various species. Since non-enterococcal fecal streptococci were not studied for their ability to produce bacteriocins, this avenue for taxonomic classification should be further explored.

Numerical taxonomy has been successfully used in clarifying relationships among the fecal, pyogenic, and viridans streptococci (64). These studies showed a large homogeneous cluster which included not only the enterococci, but also S. bovis. Another discrete cluster was composed of other members of the viridans and the pyogenic group. This second cluster showed low cross relationships with all the enterococcal strains, including S. bovis. Raj noted that extension should be made of his preliminary findings to a detailed study of more strains of the streptococcal species to reevaluate the so-called fecal, pyogenic, and viridans groups on a sound taxonomic basis. Within the fecal group, this method could be used to define the so-called "odd biotypes" which are isolated so frequently (35), and to examine the etiology of organisms isolated from the environment.

The most useful taxonomic methods still remain the oldest ones: biochemical and physiological reactions, and to a lesser degree,

serology. Many problems remain in using such reactions as the basis for identification of fecal streptococci. In 1955, Shattock pointed out that, although group D streptococci comprise several well defined species and variants, not all of them can be assigned a species name. He felt that there were not clear-cut lines of demarcation between various species, and those that cannot be fitted into a given species must remain anonymous (66). However, Papavassiliau (61) and Deibel (16) both observed that, in identifying an organism, one must depend on a spectrum of characteristics, and that failure to comply in a few specific tests should not constitute sufficient grounds to negate speciation. Hartman further clarified the issue when he suggested flexibility whereby one could specify a certain number or percentage of features which should be fulfilled in order for a microorganism to be designated as a particular species (31).

Andrewes and Horder first identified S. faecalis as the predominant streptococcus isolated from human feces (2). Important attributes of this species, in addition to Sherman's criteria for enterococci, include the ability to cause complete reduction of litmus milk before formation of a hard, acid clot, fermentation of sorbitol (68) and, under anaerobic conditions, glycerol fermentation (17), resistance to 0.04% potassium tellurite, and ability to reduce 2,3,5-triphenyl tetrazolium chloride (4).

Sherman originally placed strains which were hemolytic or proteolytic into separate species, S. zymogenes and S. liquefaciens (67). Because it was later found that these two species differed from S.

faecalis only in their proteolytic and hemolytic abilities, they were assigned varietal status. Since then, the B-hemolysin has been found to be unstable (17) and plasmid dependent (34). On this basis it was suggested that the varietal status be dropped for S. faecalis var. zymogenes (17).

Streptococcus faecalis var. liquefaciens was originally considered the only fecal streptococcus with proteolytic activity. It has been observed that a more sensitive method of proteolysis detection (17) shows that both S. faecalis and S. faecium have some proteolytic activity. Nevertheless, since the hydrolysis of gelatin, as detected by the tube method, does have some value in differentiating those strains of S. faecalis which have sanitary significance (24), it appears that the S. faecalis var. liquefaciens variety should be retained.

It is fascinating to observe the varied status which S. faecium and S. durans have held. Orla-Jensen in 1919 first introduced S. faecium as a species separate from S. glycerinaceus (later identified as S. faecalis) (60). When Sherman published his classification of the streptococci, he included S. durans among the enterococci, but considered the fermentation reactions of S. faecalis and S. faecium too varied to merit consideration of S. faecium as a species (67,68). Then, in 1963, Deibel et al. proposed that S. faecium was a species independent from S. faecalis, but that S. durans should be designated a variety of S. faecium (17). He justified this on the grounds that S. faecalis and S. faecium differed in their fermentation reactions of arabinose, melibiose, melezitose, and glycerol, their utilization of citrate and

gluconate as an energy source, and reduction of tetrazolium. Streptococcus durans was believed to differ only in a couple of reactions. Now, S. durans is differentiated from S. faecium by its failure to ferment sucrose, mannitol, or arabinose, decreased reduction of 2,3,5-triphenyl tetrazolium chloride (4), and presence of a B-hemolysin. Numerical taxonomy studies by Raj and Colwell supports the definition of S. durans as a species separate from S. faecium (64).

Another reason for confusion was the occurrence of strains with properties intermediate between S. faecalis and S. faecium. Yellow pigmented streptococci have frequently been reported in the literature as residents of plants (54). In 1968, Mundt and Graham made a study of these bacteria, and found them different from S. faecalis and S. faecium in physiology, ecology, and motility (53). They named this organism S. faecium var. casseliflavus. With S. faecalis it shares the ability to reduce TTC, resistance to potassium tellurite, and ability to ferment cellobiose, dextrin, maltose, mannose, and sorbitol. Like S. faecium, arabinose, melibiose, and salicin are fermented, while melezitose is not. Unlike either organism, it ferments raffinose, xylose, and inulin. Other characteristics peculiar to this organism are the yellow pigment and motility.

Streptococcus avium shares many of the characteristics with the enterococci. However, it fails to grow in 0.1% methylene blue milk, or to hydrolyze arginine. It is also characterized by its ability to ferment a wide variety of polyols, and to grow in pH 10 medium with sorbose as the energy source (59).

Streptococcus bovis, S. equinus, S. salivarius, and S. mitis all belong to Sherman's viridans group. While they do grow at 45C, they cannot grow at 10C, in 6.5% NaCl, at pH 9.6, in 0.1% methylene blue milk, nor can they produce ammonia from peptone.

Streptococcus bovis was named and described by Orla-Jensen in 1919 (60). Its characteristics include fermentation of arabinose, raffinose, starch, and inulin. It is also characterized by its ability to hydrolyze starch to reducing sugars, and to utilize ammonium salts as a sole source of nitrogen.

Streptococcus equinus was first observed as a non-lactose fermenting contaminant from air (2). Since a large part of the organic pollution in the air of cities was due to horse dung, it was suspected as the source of these organisms. Streptococcus equinus, in fact, makes up a majority of the total bacterial flora of the horse intestine. It is characterized by a high minimum temperature of growth, inability to grow in litmus milk, fermentation of glucose, fructose, galactose and maltose, and inability to ferment arabinose, xylose, lactose, mannitol, glycerol or sorbitol (32).

Streptococcus salivarius and S. mitis are human buccal streptococci which enter the digestive tract and populate feces in low numbers (13, 65,67,69). The principal characteristics of S. salivarius include production of extracellular levan when grown on 5% sucrose (57), fermentation of glucose, maltose, lactose, sucrose, raffinose, inulin, and salicin (65). Streptococcus mitis is differentiated from S. salivarius by its inability to produce polysaccharide from 5% sucrose, or to ferment raffinose or inulin (69).

A summary of the chronology of the taxonomy of the fecal streptococci is presented in Table 2.

Fecal streptococci are clinically significant as a chief cause of subacute bacterial endocarditis (40,41,73), urinary tract infections (8,12,29,40,75), wound infections (29), suppurative infections such as peritonitis and meningitis (75), and dental caries (58).

Both the viridans and enterococcal groups of streptococci are included as the main agents of subacute bacterial endocarditis (73). This is a condition caused by lodgment of the Streptococcus in an abnormal heart or in valves damaged previously by rheumatic fever, and may follow extraction of a tooth, or an infection of the tonsils, teeth, or sinuses (21).

Close to 50% of all clinical enterococcal isolates come from urinary tract infections (12). Enterococci are responsible for approximately 10% of all isolates from such infections (8).

Group D meningitis is rare, and tends to occur in patients already seriously ill, especially those with an underlying enterococcal endocarditis, urinary tract disease, or neurosurgical interventions in whom anatomic central nervous system defects are present (7).

In 1965, Krantz classified streptococci associated with domestic animals. He found Group D streptococci associated with septicemia, pneumonia, enteritis, vaginitis, infertility, mastitis, and hog cholera. These bacteria were also unusually prevalent in cases of infertility (38).

To gain insight into the relative pathogenicity of fecal streptococci, Bump and Hardy, in independent studies, found quite similar results when they speciated all group D streptococci from various clinical origins (12,29). Streptococcus faecalis was the predominant organism isolated from all clinical cultures (60-80%). Streptococcus faecium was isolated much less frequently and on the same order of magnitude as S. bovis and S. avium (2-5%). Bump was the first to report human isolates of S. faecium var. casseliflavus. He also found S. equinus to be a formidable pathogen, comprising 17% of all clinical isolates of group D streptococci. Streptococcus salivarius and S. mitis were also isolated with relatively high frequency in Hardy's study, even though Safford et al. reported in 1937 that these organisms had no apparent pathogenicity for rabbits or mice (65). Hardy (32) found that S. mitis was isolated approximately seven times as often as S. salivarius from all clinical sources.

The use of streptococci as indicators of fecal pollution dates back to 1902 (76) and as a means of differentiating human from other mammalian sources of contamination, back to 1910 (77). Inadequate means of detection and enumeration prevented application of this knowledge to sanitary microbiology. It was believed that relatively low numbers of fecal streptococci could be isolated from sewage or polluted water. Indeed, it had been reported that E. coli in river water samples outnumbered enterococci by a factor of 50 or more (42). It wasn't until 1948, when Roth reported on a practical medium, an azide dextrose broth, that selective enumeration of enterococci from a grossly contaminated environment was possible without concomitant excessive inhibition of

the enterococci themselves (15). After this, research into new methods and media for recovery of fecal streptococci fluorished.

By 1972, 68 different media formulations were available for the selective isolation or enumeration of these organisms (30). One reason for this abundance is the wide range of selective agents available. Among the more successful ones are sodium azide, thallium salts, tetrazolium salts, potassium tellurite, and bile salts. Other agents include sodium chloride, penicillin, phenethyl alcohol, high pH, selenite, tetrathionate, and elevated incubation temperature. In most media preparations, two or more of these ingredients are used in combination for enhanced selectivity (30).

The two media now most frequently used are KF streptococcus agar, which uses 0.04% sodium azide and 0.01% 2,3,5-triphenyltetrazolium chloride as selective and differential agents, and Pfizer selective enterococcus agar, which contains 1% bile salts, 0.025% sodium azide, and 0.1% esculin (1,33,36).

Pavlova et al., in 1972, found that of five popular media preparations, KF streptococcus agar and PSE agar yielded the highest recovery of fecal streptococci while showing the lowest percentage of nonfecal streptococci (63). They found little difference in the proportions of non-enterococcal fecal streptococci isolated, but because of the shorter incubation time (24 as opposed to 48 hours), they preferred PSE agar. Dutka and Kwan concurred with these results in 1978 (18).

In a more comprehensive study, Brodsky and Schiemann found that 99% of all tanned colonies on PSE agar were confirmed as fecal streptococci; of total colonies, (tanned and nontanned) 90% were confirmed as fecal streptococci. KF streptococcus agar, on the other hand, had an overall confirmation rate of only 65% while only 83% of the typical red to pink colonies were confirmed as fecal streptococci. So while KF agar recovered significantly greater numbers of organisms, PSE agar was more selective for fecal streptococci (11).

It should be pointed out that, to date, there is no medium designed for total recovery of non-enterococcal fecal streptococci. There are several reasons for this. First, many media were designed with the clinical laboratory in mind (19). Because of the highly resistant nature of enterococci, clinicians require rapid differentiation of these organisms from other streptococci. In addition, many sanitary bacteriologists have ignored the significance of the non-enterococcal streptococci in differentiating human from animal pollution. Finally, there is the problem of finding an appropriate selective agent for the viridans streptococci which doesn't select more for the enterococci.

Many different studies of intestinal flora have been performed over the years, in spite of Jordan's statement, "whenever a man gets the idea that he is going to work out the bacteriology of the intestinal tract of any mammal, the time has come to have him quietly removed to some suitable institution" (48). Perhaps the reason why Jordan found such an undertaking so formidable was that there is such wide variation

in the relative numbers and kinds of microorganisms, even among hosts of the same species. This is particularly true for the streptococci, each species of which is never invariably present in a given host. However, to understand the ecology of the fecal streptococci, the factors influencing the populations of microorganisms in feces need to be understood.

Although individuals tend to have a fairly stable fecal population from one sampling period to another (28,78), within a given species there is a large variation in relative numbers of organisms present (28,44). Increasing age (72), type and amount of food consumed (72), amount of complex carbohydrates in the diet (44), and fecal moisture content (24) have been implicated in the variety and amount of fecal flora. It appears from Gorbach's report that the consumption of a standardized diet among a group of individuals does not produce a standardized fecal flora; indeed, the intestinal microbial diversity among such a group of people had as much variation as another group eating entirely dissimilar, random diets (28).

Streptococci have been found to constitute the fourth largest group of bacteria present in the intestines of humans (28), their concentration ranging from 10^6 to 10^{11}/gm wet wt of feces (44). Cooper and Ramaden found typical S. durans and S. faecalis to comprise 4% and 40%, respectively, of the human streptococcal flora, whereas S. liquefaciens and S. zymogenes made up 29% (49). The enterococci constitute an estimated 76% and the S. salivarius group 16% (6,35). At times, S. salivarius and S. mitis comprise the predominant streptococcal flora of

the human intestine, but they are not invariably present (69). While in most surveys no S. bovis or S. equinus have been isolated from human feces, occasionally these organisms may show up in trace amounts (6).

In 1963, Mundt reported that only 71% of mammals, 86% of reptiles, and 32% of birds possessed enterococci (50). He determined that enterococci were generally not recovered from strongly herbivorous animals or from subsurface ground dwellers, but that they were commonly encountered in those animals with a more varied diet. Kenner has characterized the feces of quadrupeds as harboring S. bovis, S. equinus, and a few enterococci (35). Bartley and Slanetz enumerated enterococci in animals, and found them to comprise 10% of the fecal streptococci in swine, 12% in cows, 25% in sheep, and 62% in fowl (4). Geldreich encountered significant numbers of S. bovis and S. equinus in cows, sheep, horses, pigs, ducks, dogs, and rodents. Only about 1% of the streptococci in chickens, turkeys, or cats were S. bovis or S. equinus. He found S. faecalis var. liquefaciens to be present in all specimens studied (26). In no animal other than human beings was S. salivarius or S. mitis isolated.

One of the major disadvantages of using enterococci as indicators of fecal pollution is their prevalence in environments free of recent fecal contamination. Enterococci have been isolated from insects (27), plants (49,54,50,27), and agricultural soils (37,47,49,74) with high frequency. Fifty-eight percent of insects studied (27), 70% of agricultural soils, and 28% of plants (49) harbor enterococci. When present in soils, the concentration ranges from 10^1 to 10^3/gram (49).

In plants, the average concentration was less than 10^3/gram. When more remote environments were investigated, these numbers were considerably decreased. Only 2.2% of remote soils contained enterococci, and these were attributed to chance animal or bird droppings, or surface or subsurface drainage (47). The number of plants harboring enterococci decreased to 14% (50). The percent of enterococcal recovery in such an environment was influenced adversely by dense forest cover and by increase in elevation, but increased directly with rising seasonal temperature. The origin of these organisms on vegetation can be attributed to wildlife and insects, but once present on plants, are capable of limited reproduction. However, they must be reintroduced during each growing season (56).

In 1967, Mundt further elucidated the species of enterococci prevalent on raw vegetables. Streptococcus faecalis comprised 8.3% of the streptococcal population, 28.8% were S. faecalis var. liquefaciens, 15.4% were S. faecium, and 28.2% were a yellow pigmented enterococcus, in all probability S. faecium var. casseliflavus, which Mundt defined in 1968 to be a resident of plants which is only rarely isolated from feces (53,54).

It has been found that isolates of S. faecalis from plants and humans are dissimilar, but only in random deviations in various biochemical tests (51). This lack of a pattern in plant isolates reflects fluctuating environmental conditions. However, non-human isolates tend to form a soft, flowing rennet curd in litmus milk, followed by

stratiform peptonization. Human isolates, on the other hand, form hard, acidic curds frequently followed by acid proteolysis (51).

While this is certainly a significant contribution, it does not account for the origin of other enterococci, since they are known to have litmus milk reactions resembling those reported for plant-originating S. faecalis (3).

Survival of fecal streptococci in the environment depends on factors such as temperature (25,37), organic matter content (43), availability of certain salts (24), toxic metallic ions or unfavorable pH (26), moisture content (37), and direct exposure to sunlight (74). Once enterococci are introduced into a system, they tend to persist a long time, but don't multiply (26). They have been found to survive in soil for as long as 142 days (74).

Species variation plays a large part in their survival. For example, most of the enterococci are recovered from vegetation and soils in the late summer, whereas S. faecium var. casseliflavus thrives during the spring and early summer months and again in midfall (53). Enterococci have an average half-time in well water of 22 hours, compared to 17 hours for coliforms. However, S. equinus and S. bovis have 50% die-off within 10 and 4 hours, respectively. In general, enterococci survive about the same or somewhat longer than the pathogens, Salmonella and Shigella (46). Geldreich has found that enterococci persisted longer than coliforms or Salmonella. All organisms he tested, except S. bovis, declined more rapidly at 20C than at 10C. Streptococcus bovis persisted for less than a day at 10C, but for greater than 3 days at 20C.

The use of fecal streptococci to define sources of contamination has been retarded because of undue emphasis on the S. faecalis group (enterococci) with little or no regard for other streptococcal strains present in the guts of humans, mammals, or birds (35). While the enterococci, with their more resistant nature, are very wide spread in the environment, the other fecal streptococci are limited to the feces of warm-blooded animals. Streptococcus bovis is found in many mammals, but absent in humans. Streptococcus equinus is restricted to the gut of the horse (24). Because of this, they are both particularly useful in pollution investigations of cattle feedlot runoff, farmland drainage, discharges from meat- and poultry-processing plants, and dairy farm operations (24). Because they are so sensitive to environmental exposure, they are useful indicators only of recent animal contamination (24). On the other hand, humans are the only mammals known to harbor S. salivarius and S. mitis. Isolation of these organisms can be interpreted as evidence of recent human fecal contamination (13,22,23, 35,63).

In 1955, Litsky showed a positive correlation coefficient of 0.84 between fecal coliforms and fecal streptococci enumerated from river water samples of varying degrees of pollution (42). More than twenty years later, these results were confirmed by Davis, who found a correlation coefficient between these two indicators to be 0.81. Since fecal coliforms represent the certain presence of recent fecal contamination (27), a high correlation between these two indicators justifies the use of fecal streptococci as indicators of recent fecal pollution.

However while fecal coliforms and fecal streptococci are good indicators, they do not necessarily indicate human pollution.

Any indicator needs to predict the presence of dangerous pathogens. Smith has found fecal coliforms and fecal streptococci to have approximately the same median value for those instances when Salmonellae were isolated from surface waters (71). Davis discovered that fecal streptococci were statistically even better predictors of pathogens in stormwaters than fecal coliforms (15). In addition, the disappearance rate of fecal streptococci more closely parallels that of viruses than does the dieoff rate of coliforms. Therefore, one could more closely approximate the concentration of viruses in a body of water by using streptococcal rather than coliform concentrations (14).

Since 1955, it has been known that sewage effluent had a higher fecal coliform: fecal streptococcus ratio than did surface waters (42). In 1967, Geldreich found that the ratio of fecal coliforms to fecal streptococci was greater than 4.4 in human feces and domestic wastewaters, while it was less than 0.7 in animal feces and stormwater (26).

Several factors can affect the relative concentrations of these two indicators. Once diffused into receiving streams, ecological forces alter the interrelationships between these indicator systems. As flow-time down-stream increases, bacterial populations within the pollution indicator groups can be reduced drastically or altered with selective adaptation to the water environment by only a few vigorous strains (24). The makeup of the streptococcal population also affects the ratio following exposure to the environment. The ratio for human feces is

found to decrease from 4.2 to 2.2 after four days in well water. The ratio from bovine feces increases from 0.25 to 2.0 in that period. However, for species with a lower population of S. bovis, the ratio remains fairly constant. This is due to the varying persistence of the enterococci and other fecal streptococci (20, 46). Again, investigators have not considered the utility of non-enterococci for estimating specific origin(s) of non-point pollution.

For this reason, the use of a ratio relationship for stream samples is valid only during the initial 24 hours of travel downstream. After this time, one could mistakenly assume the origin to be mixed animal and human contamination. Because of the lack of sanitary significance of S. faecalis var. liquefaciens, which tends to dominate the streptococcal population at low densities in surface waters, Geldreich also cautions against interpreting the fecal coliform: fecal streptococcus ratio when the fecal streptococcal densities do not exceed 100/100 ml (24, 26).

Table 1. Major Taxonomic Divisions of the Fecal Streptococci.

Enterococci

S. faecalis
S. faecalis var. liquefaciens
S. faecalis var. zymogenes
S. faecium
S. faecium var. casseliflavus
S. durans
S. avium (also group Q)

Group D

Viridans

S. bovis
S. equinus

S. mitis
S. salivarius (some group K)

Table 2. Chronology of the Taxonomy of the Fecal Streptococci

1874 Streptococci were first described by Billroth.

1905 Gordon applied fermentation tests to the streptococci in a broad and systematic manner.

1906 Andrewes and Horder reported on the origin of several species of streptococci:

1. Streptococcus faecalis as the predominant streptococcus from human feces
2. Found S. equinus to be the chief microorganism in horse dung
3. Named the predominating human mouth streptococci, S. salivarius and S. mitis.

1919 Orla-Jensen first suggested that S. faecium is a species separate from S. faecalis. He also identified S. bovis as the largest component of the streptococcal flora of the bovine feces.

1933 Lancefield reported her serological classification of the streptococci.

1937 Sherman proposed a broad classification scheme dividing the streptococci into four major taxonomic groups. In addition, he specifically suggested that S. faecium and S. faecalis are the same, whereas S. durans is a separate species.

1963 Deibel suggested that variety status of S. faecalis var. zymogenes be dropped because hemolysin production is an unstable characteristic. He further elucidated the taxonomy of the enterococci by considering S. faecium a species different from S. faecalis, but that S. durans is a variety of S. faecium.

1966 Raj affirmed that S. faecium, S. faecalis, and S. durans are all separate species, based on the results of numerical taxonomy studies.

1968 Mundt and Graham named and described S. faecium var. casseliflavus.

Literature Cited

1. American Public Health Association. 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Inc., New York.

2. Andrewes, F. W. and T. J. Horder. 1906. A study of the streptococci pathogenic for man. Lancet. 2:708-713, 775-782, 852-855.

3. Ator, Linda Louise and Marvin J. Starzyk. 1976. Distribution of group D streptococci in rivers and streams. Microbios. 16:91-104.

4. Barnes, Ella. 1956. Tetrazolium reduction as a means of differentiating Streptococcus faecalis from Streptococcus faecium. J. Gen. Microbiol. 14:57-68.

5. Barnes, Ella M. 1964. Distribution and properties of serological types of S. faecium, S. durans and related strains. J. Appl. Bact. 27:461-470.

6. Bartley, C. H. and L. W. Slanetz. 1960. Types and sanitary significance of fecal streptococci isolated from feces, sewage, and water. Am. Jour. Public Health. 50:1545-1552.

7. Bayer, Arnold S., James S. Seidel, Thomas T. Yoshikawa, Bascom F. Anthony, and Lucien B. Grege. 1976. Group D enterococcal meningitis. Arch. Intern. Med. 136:883-886.

8. Blazevic, D., Jr., J. E. Stemper, and J. M. Matsen. 1972. Organisms encountered in urine cultures over a 10-year period. Appl. Microbiol. 23:421-422.

9. Brock, Thomas D. 1964. Host range of certain virulent and temperate bacteriophages attacking group D streptococci. J. Bacteriol. 88:165-171.

10. Brock, Thomas D., Barbara Peacher, and Deborah Pierson. 1963. Survey of the bacteriocins of enterococci. J. Bacteriol. 86:702-707.

11. Brodsky, M. H. and D. A. Schiemann. 1976. Evaluation of Pfizer Selective Enterococcus and KF Media for recovery of fecal streptococci from water by membrane filtration. Appl. Environ. Microbiol. 31:695-699.

12. Bump, C. M. 1977. Isolation of S. equinus from non-respiratory sources in children. J. Clin. Microbiol. 6:433-434.

13. Clausen, E. M., B. L. Green, and Warren Litsky. 1977. Fecal streptococci: indicators of pollution In Bacterial indicators/ health hazards associated with water. ASTM STP635. A. W. Hoadley and B. J. Dutka, Eds. Am. Society for Testing and Materials, p. 247-264.

14. Cohen, Judith and Hillel I. Shuval. 1973. Coliforms, fecal coliforms, and fecal streptococci as indicators of water pollution. Water, Air, and Soil Pollution. 2:85-95.

15. Davis, E. M., D. M. Casserly, and J. D. Moore. 1977. Bacterial relationships in storm-waters. Water Resources Bulletin. 13:895-905.

16. Deibel, R. H. 1964. The group D streptococci. Bacteriol. Rev. 28:330-366.

17. Deibel, R. H., Donald E. Lake, and C. F. Niven, Jr. 1963. Physiology of the enterococci as related to their taxonomy. J. Bacteriol. 86:1275-1282.

18. Dutka, B. J., and K. K. Kwan. 1978. Comparison of eight media-procedures for recovering faecal streptococci from water under winter conditions. J. Appl. Bacteriol. 45:333-340.

19. Facklam, R. R. 1973. Comparison of several laboratory media for presumptive identification of enterococci and group D streptococci. Appl. Microbiol. 26:138-145.

20. Feachem, R. 1975. An improved role for faecal coliform to faecal streptococci ratios in the differentiation between human and non-human pollution sources. Water Res. 9:689-690.

21. Frobisher, Martin, Lucille Sommermeyer, and Robert Fuerst. Microbiology in health and disease. 12th ed. 1969. W. B. Saunders Co., Philadelphia, Pennsylvania.

22. Galvani, Mary Moriarty. 1974. Fecal contamination - the water analyst's responsibility. Part 1. Water and Sewage Works. 121:66-69.

23. Galvani, Mary Moriarty. 1975. Fecal contamination - the water analyst's responsibility. Part II. Water and Sewage Works. 122:68-70.

24. Geldreich, Edwin E. 1976. Fecal coliform and fecal streptococcus density relationship in waste discharges and receiving waters. CRC Critical Reviews in Environ. Control. 6:349-369.

25. Geldreich, E. E., L. C. Best, B. A. Kenner, and D. J. Van Donsel. 1968. The bacteriological aspects of stormwater pollution. J. Water Pollut. Control Fed. 40:1861-1862.

26. Geldreich, Edwin E., and Bernard A. Kenner. 1969. Concepts of fecal streptococci in stream pollution. J. Water Pollut. Control Fed. 41:R336-R352.

27. Geldreich, E. E., B. A. Kenner, and P. W. Kabler. 1964. Occurrence of coliforms, fecal coliforms, and streptococci in vegetation and insects. Appl. Microbiol. 12:63-69.

28. Gorbach, Sherwood L., Laila Nahas, Phillip I. Lerner, and Louis Weinstein. 1967. Studies of intestinal microflora. I. Effects of diet, age, and periodic sampling on numbers of fecal microorganisms in man. Gastroenterology. 53:845-855.

29. Hardy, Margaret A., Harry P. Dalton, and Marvin J. Allison. 1978. Laboratory identification and epidemiology of streptococcal hospital isolates. J. Clin. Microbiol. 8:534-544.

30. Hartman, Paul A., George W. Reinbold, and Devi S. Saraswat. in Advances in Applied Microbiology. (W. W. Umbreit, ed.) Academic Press, N.Y., N.Y., Vol. 8. p. 253-289. 1966.

31. Hartman, P. A., G. W. Reinbold, and D. S. Saraswat. 1966. Indicator organisms - a review. I. Taxonomy of the fecal streptococci. Int. J. Syst. Bacteriol. 16:197-222.

32. Hodge, H. M., and J. M. Sherman. 1937. *Streptococcus equinus*. J. Bacteriol. 33:283-289.

33. Isenberg, Henry D., Dorothy Goldberg, and Jane Sampson. 1970. Laboratory studies with a selective enterococcus medium. Appl. Microbiol. 20:433-436.

34. Jacob, A. E., G. J. Douglas, and S. J. Hobbs. 1975. Self-transferrable plasmids determining the hemolysin and bacteriocin of *S. faecalis* var. zymogenes. J. Bacteriol. 121:863-872.

35. Kenner, B. A., H. F. Clark, and P. W. Kabler. 1960. Fecal streptococci. II. Quantification of streptococci in feces. Am. J. Public Health. 50:1553-1559.

36. Kenner, B. A., H. F. Clark, and P. W. Kabler. 1961. Fecal streptococci. I. Cultivation and enumeration of streptococci in surface waters. Appl. Microbiol. 9:15-20.

37. Kibbey, H. J., C. Hagedorn, and E. L. McCoy. 1978. Use of fecal streptococci as indicators of pollution in soil. Appl. Environ. Microbiol. 35:711-717.

38. Krantz, George E., and Howard W. Dunne. 1965. An attempt to classify streptococcic isolates from domestic animals. Am. J. Veterinary Res. 26:951-959.

39. Lancefield, R. C. 1933. A serological differentiation of human and other groups of hemolytic streptococci. J. Exptl. Med. 57:571-595.

40. Lennette, Edwin H., Earle H. Spaulding, and Joseph P. Truant. (eds.) 1974. Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C. p. 96-108.

41. Lerner, P. I., and L. Weinstein. 1966. Infective endocarditis in the antibiotic era. New England J. Med. 274:199-206.

42. Litsky, Warren, Walter L. Mallmann, and C. W. Fifield. 1955. Comparison of the most probable numbers of Escherichia coli and enterococci in river waters. Am. J. Public Health. 45:1049-1053.

43. Mallmann, W. L., and Warren Litsky. 1951. Survival of selected enteric organisms in various types of soil. Am. J. Public Health. 41:38-44.

44. Mata, Leonardo J., Celina Carrillo, and Elba Villatoro. 1969. Fecal microflora in healthy persons in a preindustrial region. Appl. Microbiol. 17:596-602.

45. Matson, Ernest A., Sally G. Hornor, and John D. Buck. 1978. Pollution indicators and other microorganisms in river sediment. J. Water Pollut. Control Fed. 50:13-19.

46. McFeters, Gordon A., Gary K. Bissonnette, James J. Jezeski, Carole A. Thomson, and David G. Stuart. 1974. Comparative survival of indicator bacteria and enteric pathogens in well water. Appl. Microbiol. 27:823-829.

47. Medrek, T. F., and W. Litsky. 1960. Comparative incidence of coliform bacteria and enterococci in undisturbed soil. Appl. Microbiol. 8:60-63.

48. Miller, C. P. 1959. Protective action of the normal microflora against enteric infection: an experimental study in the mouse. Univ. Mich. Med. Bull. 25:272-279.

49. Mundt, J. O. 1961. Occurrence of enterococci: bud, blossom and soil studies. Appl. Microbiol. 9:541-544.

50. Mundt, J. Orvin. 1963. Occurrence of enterococci on plants in a wild environment. Appl. Microbiol. 11:141-144.

51. Mundt, J. O. 1973. Litmus milk reaction as a distinguishing feature between S. faecalis of human and non-human origins. J. Milk Food Technol. 36:364-367.

52. Mundt, J. Orvin, J. H. Coggin, and L. F. Johnson. 1962. Growth of S. faecalis var. liquefaciens on plants. Appl. Microbiol. 10:552-555.

53. Mundt, J. O., and W. F. Graham. 1968. Streptococcus faecium var. casseliflavus, nov. var. J. Bacteriol. 95:2005-2009.

54. Mundt, J. O., W. F. Graham, and I. E. McCarty. 1967. Spherical lactic acid-producing bacteria of southern-grown raw and processed vegetables. Appl. Microbiol. 15:1303-1308.

55. Myrvik, Quentin N., Nancy N. Pearsall, and Russel S. Weiser. 1974. Fundamentals of medical bacteriology and mycology. Lea and Febiger, Philadelphia, Pennsylvania.

56. Nivin, C. F. Jr., R. C. Lancefield, R. H. Deibel, I. C. Gunsalus, and P. A. Hartman. 1964. The enterococci. Bact. Proc. p. xvi-xviii.

57. Nivin, Charles F., Karl L. Smiley, and J. M. Sherman. 1941. The production of large amounts of a polysaccharid by Streptococcus salivarius. J. Bacteriol. 41:479-484.

58. Nolte, William A. Oral Microbiology. 1968. C. V. Mosley Co. Saint Louis, Missouri.

59. Nowlan, Sandra Simpson, and R. H. Deibel. 1967. Group Q Streptococci: I. Ecology, serology, physiology, and relationship to established enterococci. J. Bacteriol. 94:291-296.

60. Orla-Jensen, S. 1919. The lactic acid bacteria. A. F. Host, Copenhagen, Denmark.

61. Papavassiliou, J. 1962. Species differentiation of Group D streptococci. Appl. Microbiol. 10:65-69.

62. Pavlova, Maria T., Elizabeth Beauvais, Francis T. Brezenski, and Warren Litsky. 1972. Fluorescent-antibody techniques for the identification of group D streptococci: direct staining method. Appl. Microbiol. 23:571-577.

63. Pavlova, Maria T., Francis T. Brezenski, and Warren Litsky. 1972. Evaluation of various media for isolation, enumeration and identification of fecal streptococci from natural sources. Health Lab. Sci. 9:289-298.

64. Raj, H., and R. R. Colwell. 1966. Taxonomy of enterococci by computer analysis. Can. J. Microbiol. 12:353-362.

65. Safford, C. E., J. M. Sherman, and H. M. Hodge. 1937. Streptococcus salivarius. J. Bact. 33:263-274.

66. Shattock, P. M. F. 1955. The identification and classification of Streptococcus faecalis and some associated streptococci. Ann. Inst. Pasteur Lille. 7:95-100.

67. Sherman, James M. 1937. The streptococci. Bact. Rev. 1:1-97.

68. Sherman, J. M., J. C. Mauer, and Pauline Stark. 1937. Streptococcus fecalis. J. Bacteriol. 33:275-282.

69. Sherman, J. M., C. F. Nivin, Jr., and K. L. Smiley. 1943. Streptococcus salivarius and other non-hemolytic streptococci of the human throat. J. Bacteriol. 45:249-263.

70. Shockman, G. D., and H. D. Slade. 1964. The cellular location of the streptococcal group D antigen. J. Gen. Microbiol. 37:297-305.

71. Smith, R. J., R. M. Twedt, and Linda Krusel Flanigan. 1973. Relationships of indicator and pathogenic bacteria in stream waters. J. Water Pollut. Control Fed. 45:1736-1745.

72. Smith, William. 1962. The development of the bacterial flora of the faeces of animals and man. The changes that occur during ageing. J. Appl. Bact. 24:235-241.

73. Thomas, Clayton L. 1970. Taber's cyclopedic medical dictionary. F. A. Davis Company, Philadelphia, Pennsylvania. p. E-30.

74. Van Donsel, Dale J., Edwin E. Geldreich, and Norman A. Clarke. 1967. Seasonal variation in survival of indicator bacteria in soil and their contribution to stormwater pollution. 15:1360-1370.

75. Whaler, S. M., and G. E. Foley. 1943. A note on non-group A streptococci associated with human infection. J. Bacteriol. 46:391-392.

76. Winslow, C. E., and M. P. Hunnewell. 1902. Streptococci characteristics of sewage and sewage-polluted waters. Science. 15:827.

77. Winslow, C. E. A., and G. T. Palmer. 1910. A comparative study of intestinal streptococci from the horse, the cow, and man. J. Infectious Dis. 7:1.

78. Zubrzycki, Leonard, and Earle H. Spaulding. 1962. Studies on the stability of the normal human fecal flora. J. Bacteriol. 83:968-974.

Chapter 2.

Origin and Extent of Non-point Surface Water Pollution Resulting from Rural Population Growth

Karla Lamka and Ramon J. Seidler

Department of Microbiology, Oregon State University
Corvallis, Oregon 97331

Proofs, reprints, and other correspondence to:

Dr. Ramon J. Seidler
Department of Microbiology
Oregon State University
Corvallis, Oregon 97331

Technical Paper No. , Oregon Agricultural Experiment Station.

Abstract

The origin and extent of surface water pollution resulting from rural population growth was analyzed. It was found that total coliforms and fecal bacterial counts in a stream originating in a protected watershed increased ten-fold as that stream emerged into a populated valley. Only during the "first flush" effect of a storm event did the surface waters in the forest exceed suggested recreational water standards of 200 fecal coliforms per 100 ml. The only time that these standards were not exceeded in the downstream portion of the creek was during the winter months when the steady state flow rate was much higher than in the summer. Under all weather conditions, numbers of fecal coliforms were found to correlate very well with numbers of fecal streptococci and stream turbidity. Only during the first flush of heavy precipitation did the relative changes in fecal coliform densities correlate with stream flow rate.

Distinct differences in the biotypes of streptococci could be demonstrated between the protected area and the area receiving runoff affected by man's activity. In the runoff from the nonprotected area, a large proportion of the biotypes were Streptococcus salivarius and S. mitis, organisms found in this and other studies to arise only from human fecal contamination. Only trace amounts of these two species were encountered on one occasion in the limited access forest. The isolation of S. bovis was indicative of domestic livestock and wildlife pollution. Enterococci were found to be ubiquitous in distribution, and so of no use in defining the origin of non-point

contamination. The fecal coliform to fecal streptococcus ratio, useful in interpreting the origin of point sources of pollution, was nearly always less than 1.0 in the creek, even when the human biotype was prevalent, and so of no use in defining non-point pollution. It was concluded that the large increase in bacterial counts below the protected watershed could be attributed to the use of septic tanks in soils unsuitable for use as drainfields, and to the presence of grazing livestock.

Introduction

The Federal Water Pollution Control Act Amendments (PL-92-500) call for the documentation of the origins and extent of surface water pollution resulting from rural population growth demands. Because in rural areas there are usually no known point sources of pollution, control of non-point sources is crucial if fishable and swimmable surface waters are to be achieved by 1983, as mandated by the amendments.

Previous studies have demonstrated an increase in surface water pollution that results from man's activity (21, 29). However, a quantitative assessment of the impact on surface waters of a modern rural housing development has not been established. In rural areas, several factors affect the impact of runoff on surface water quality: soil types, climate, type of land use, topography, extent of human utilization and animal density (29). The purpose of this study was to analyze the levels and types of fecal indicators present in both protected and non-protected regions of the same surface water in a rural community.

Storm events are particularly important when assessing the impact of non-point pollution. During rainfall conditions, both runoff and infiltration occur in the basin, resulting in an increasing and decreasing hydrograph. The increase in mass loading during rainfall and subsequent runoff conditions is due to nonpoint source effects (29). At various stations in the study area, the kinetics of water quality changes due to storm events were defined in a quantitative fashion.

Fecal streptococci have long been recognized as indicators of fecal contamination (30). However, the use of fecal streptococci to define sources of contamination has been retarded by undue emphasis on the enterococcal group with little or no regard for other streptococcal strains present in the intestines of humans and other mammals (11). Fecal coliform to fecal streptococcus ratios have been utilized for differentiation of human from non-human contamination, but this method is unsatisfactory in the absence of point sources of contamination (6,7). In this study, species identification of all fecal streptococci has proved to be the most reliable means of identifying contributing sources of fecal contamination. A new biotyping scheme consisting of easily interpreted tests was developed to efficiently differentiate the various species of fecal streptococci.

The Study Area

A map of the study area is shown in Figure 1. The Oak Creek watershed, located 11 kilometers northwest of Corvallis, Oregon, covers approximately 750 hectares. The northern portion of the basin drains part of MacDonald Forest, a 4450 hectare state-owned forest used only for research activities. Overstory vegetation is 100- to 200-year-old Douglas fir, Oregon white oak, and red alder. A large population of black-tailed deer inhabits the forest, along with other fur-bearing animals.

The southern portion of the creek drains a rural community of approximately 100 households. Most houses in this development are less than ten years old. Modern zoning criteria were used in construction of the development, so that septic tanks meet modern standards, and lots are one to two acres in size for single family dwellings. Crops grown in the area are mainly hay and cereal grain. A large herd of sheep, in addition to some horses and cows, are pastured in he area during part of the year.

Slopes along the length of the creek range from 3 to 50%. Soils belong mainly to the Dixonville and Philomath series (27). The area is generally unsuitable for septic tank drainfields because of seasonally high water tables, shallow or slowly permeable soils.

The average annual precipitation is 40 to 60 inches, 70% of which falls between November and March. The frost-free season is 165 to 200 days. Average annual air temperature is 52 to 54F.

Materials and Methods

Water samples were collected in sterile, wide-mouth, 500-ml bottles, stored on ice, and processed within four hours.

Total and fecal coliforms and fecal streptococci were enumerated by filtering water samples through a 0.45 μm porosity membrane filters (Gelman). Such analyses are standard measurements of quality in surface waters (1). Pfizer Selective Enterococcus Agar (Gibco), m-Endo agar LES and mFC agar (Difco) were used to enumerate fecal streptococci, total coliforms, and fecal coliforms, respectively. Total plate count was estimated by spread plate technique on Standard Plate Count Agar (Difco), which was incubated for 48 hours at 35C. Total coliforms and fecal streptococci were incubated for 24 hours at 35C, and fecal coliforms for 24 hours at 44.5C.

Under both high flow and low flow conditions, several replicate samples were collected and analyzed to determine the variability of indicator concentrations. Standard deviations during high flow winter months was approximately 25%, whereas during low flow summer conditions, the standard deviation ranged between only 5 and 15%.

Stream flow rates were estimated using stream depth at a calibrated weir.

Storm events were monitored by collecting several water samples per day following the beginning of precipitation. Correlation coefficients (17a) between the different variables were calculated.

Because of the time and expense encountered when fecal streptococci were completely speciated using standard methods (1), an

alternative, efficient biotyping scheme was devised. This scheme consisted of growth at 10C in Brain Heart Infusion Broth (Difco), growth in 6.5% sodium chloride medium described by Qadri et al (19), starch hydrolysis on starch agar (Difco), and fermentation of lactose, L-arabinose, and D-sorbitol, using phenol red broth base with one percent carbohydrate and one tenth percent agar (Difco). Species identification for representatives of each biotype were confirmed using Facklam's scheme for identification of group D streptococci(3).

To define the source of the biotypes, samples of human, bovine, equine, ovine, cervid, and fowl feces, sewage effluent, soil, litter, and vegetation were cultured. Samples were either cultured directly onto PSE agar by spread plates of dilutions of blended samples, or enriched in azide dextrose medium (Difco), depending on the anticipated cell numbers present in the sample. To ensure that PSE agar was picking up all representatives of the fecal streptococci, including the non-enterococcal species, the isolates obtained from this medium were compared to those obtained by KF Streptococcal agar (Difco).

Results

Figure 2 illustrates the bacterial concentrations at the various sampling sites, from upstream in the uninhabited forest area (sites #9 and 8), and downstream through the rural neighborhood (sites #7-1). Topography and land use have major effects upon the bacterial levels in the creek. While the relative concentrations at each site vary from one sampling period to another, a one to two log increase after the stream emerges from the forest is always demonstrable. At this point, the watershed is no longer protected from man's activities. Immediately adjacent to site #7, several horses, cows, and goats are grazed. Bacterial levels at sites #4 and 5 vary according to the presence of a herd of sheep grazed within 200 feet of these sites during some seasons of the year. Site #3 generally has the highest bacterial concentrations in the stream, being downslope from households which all use septic drainfields as waste disposal. In addition, it is at the bottom of a valley formed by Cardwell Hills Road, down which contaminated runoff flows. Site #2 is subject to much the same type of contamination, being located in a basin rimmed by homesites. Although not illustrated in this particular sampling, this is frequently reflected by bacterial counts close to those at site #3. However, there is not the animal presence which there is surrounding site #3. Finally, site #1 usually experiences a drop in bacterial concentration, since it lies close to a mile east of the foothills so that it does not receive nearly the amount of runoff pollution experienced by the other sites. In

addition, it is not closely surrounded by grazing animals, and the concentration of homesites is much less in this area, being used more for cropland than for rural development.

In Figure 2 it should also be noted that total coliform to fecal coliform ratios are close to ten at sites #8 and 9. It can be assumed that most of the coliforms at these sites are washed out of soil and vegetation, and probably consist mainly of Enterobacter spp. and Klebsiella spp. However, from sites #7 to #3, this ratio decreases to nearly one. These areas are immediately adjacent to grazing animals, and downslope from septic drainfields. At such locations, most coliforms would originate from fecal sources, accounting for the similarity in numbers between total and fecal coliforms at these sites.

Figures 3 and 4 closely examine the seasonal variation of the bacterial quality of the creek, both in the forest and downstream at site #3. Table 2 lists the mean concentration of fecal coliforms and fecal streptococci during conditions of steady state low and high flow, and at peak loading during a storm event. The numbers in the Tables correspond to mean counts for the three flow conditions depicted in Figures 3 and 4. Bacterial concentrations are much lower under steady state flow conditions during the colder winter months than during the summer. Fecal bacteria are occasionally undetectable at site #9 during the winter period. This could be attributed to the greater dilution of organisms due to increased streamflow, the colder temperatures that do not allow for aftergrowth of organisms

following deposition and sedimentation, or a lack of available organic matter.

Storm events of sufficient intensity and duration caused dramatic increases in bacterial levels, demonstrating a "first flush" effect. This was manifested in bacterial levels closely corresponding with the rising and falling hydrograph, as in the storm event of November 19, 1978, illustrated in Figures 5 and 6. Following this peak in counts, there is a rapid decline in counts due to dilution from further runoff which results in a bacterial concentration lower than the concentrations before the start of precipitation.

However, there is a magnitude of difference in the impact of the storm on bacterial levels at the two different sites. At site #3, the fecal coliform to fecal streptococcus ratio remains close to 0.1, whereas at site #9 the fecal coliforms are slightly higher in concentration than are the fecal streptococci.

As seen in Figures 3 and 4, the size of the peak of bacterial concentrations during storm events is more closely related to the length of the dry spell preceding the storm event than the season of year or the actual amount of precipitation. This is most clearly demonstrated in late January and early February, and again in August and September. While the second storm event in these two periods of time involved a much greater level of precipitation, the first storm yielded a higher bacterial peak, having been preceded by a considerable period with no rainfall.

Table 3 lists the correlation coefficients between the various

indicators for the storm event in November of 1978. This particular storm event is quite similar to others monitored during the study period. Fecal coliforms, fecal streptococci, and turbidity are closely correlated, whereas total coliforms are only slightly correlated with these other parameters. However, the flow rate in this case has a higher correlation with the bacterial indicators than is seen in other storms. It is found that as the length of a storm event increases, the flow rate has an opposing effect on bacterial levels, causing their numbers to be diluted. In the November storm, the rainfall ended early, causing the flow rate to abate at the same pace as the numbers of bacteria.

This same phenomenon is apparent when the correlation coefficients for these variables are expanded to include data from all weather conditions. Fecal coliforms and fecal streptococci have a coefficient of 0.81 and 0.95 at sites #3 and 9, respectively, and both parameters have a strong relationship to turbidity. However, the correlation coefficients between flow rate and fecal coliforms, fecal streptococci, and optical density are all close to zero.

Seven sites along winter-run ditches that run through and across the housing development and in the forest were sampled three to five times each during the rainy season. Bacterial levels were found to closely correspond to the levels in the creek. If samples were taken from the protected area of the watershed, bacterial counts were very low. Conversely, when sampled from areas in the housing development, the bacterial concentrations were one to two logs higher

than in the forest. Also, bacterial concentrations in these seasonal streams showed the same relationship to the rising hydrograph as found in Oak Creek.

Thirty-five environmental samples from soil, vegetation, litter, and creek sediment were collected and cultured for fecal streptococci. These were collected from areas surrounding sites #3 and 9. Direct plating of these environmental samples on either PSE agar or KF Streptococcal agar was unsuccessful in isolating fecal streptococci. While it was impossible to determine the relative concentrations of all the different species present using enrichment technique in azide dextrose broth, this method was successful in enumeration of the fecal streptococcus group by a multiple tube procedure (MPN technique). At site #3, 82% of samples enriched in the broth yielded fecal streptococci, while 60% were positive from site #9. Soil samples from site #3 harbored between 2.2 and 43 fecal streptococci per 100 grams, creek sediment between 22 and 26 per 100 grams, and vegetation and litter between 0 and 1600 fecal streptococci per 100 grams. Only two out of six soil samples at site #9 yielded enterococci, their concentrations being 16 and 79 per 100 grams. Creek sediment at site #9 yielded enterococci in concentrations from 5 to 43 per 100 grams. Vegetation and litter yielded very low concentrations of 0 to 16 per 100 grams. Enterococcal biotypes (*S. faecalis*, *S. faecium*, *S. durans*, and *S. faecium* var. *casseliflavus*), were isolated with approximately equal frequency from all types of environmental samples. *Streptococcus bovis* and *S. equinus* were not

isolated from any of these samples. The S. mitis and S. salivarius biotypes were found only at site #3, in sediment samples collected beneath the stream level, and in pasture soil which received runoff directly from areas containing septic drainfields on steep slopes.

When human feces, sewage effluent, and other mammalian feces were examined, only human feces and sewage effluent contained the S. mitis and S. salivarius biotypes. Equine feces were the only samples to contain S. equinus. The main component of deer, cow, and sheep feces was S. bovis. Fowl feces contained only enterococci.

Thus, it appears that the non-enterococcal fecal streptococci, i.e., S. bovis, S. equinus, S. mitis and S. salivarius, can be extremely useful in identifying the source of fecal contamination in pollution studies. The enterococci, on the other hand, are ubiquitous, and so of limited sanitary significance. Because of this, one needs a medium that recovers the non-enterococcal fecal streptococci in maximum amounts. To ensure that PSE agar was the most suitable medium for doing this, it was compared to the other medium used commonly in sanitary microbiology, KF Streptococcal agar (1). The results of these tests are listed in Table 4, in which isolates from both raw sewage influent and from Oak Creek water samples are compared. It was found that KF Streptococcal agar recovered the non-enterococcal fecal streptococci with much less efficiency than did PSE agar. Based on these results, it was decided that PSE agar be used for enumeration and isolation of fecal streptococci during the remainder of the study.

Tables 5 and 6 summarize the distribution of streptococcal biotypes and fecal coliform to fecal streptococcus ratios from two sampling sites over a year-long period. The enterococci, S. faecium, S. faecium var. casseliflavus, S. faecalis and S. durans, were routinely isolated under all flow conditions at site #3. A similar observation was recorded for site #9, except that the incidence of S. faecium was significantly less than at site #3. During all weather conditions, the percentage composition of the enterococci fail to distinguish a possible origin in soil, vegetation, litter, or a mammalian intestinal tract.

The appearance of S. bovis was intermittent at both sites, while S. equinus was isolated occasionally from only site #3. With one exception, these organisms appeared only during periods of surface runoff. Thus, contribution to non-point pollution of the stream by ruminants is significant mainly when surface runoff is present. Otherwise, excretion directly into the stream is necessary for the appearance of these biotypes.

There is a high incidence of the S. mitis and S. salivarius biotypes at site #3, while, except for only one sample, it is absent at site #9. As with S. bovis and S. equinus, every isolation of S. mitis and S. salivarius corresponded to periods of rainfall and observable surface runoff. Isolation of a biotype unique to human feces is strong evidence of a human contribution to fecal pollution in the creek at site #3.

Fecal coliform to fecal streptococcus ratios have been used frequently in water quality studies to define sources of contamination

(6,7). A ratio greater than four indicates human contamination, less than 0.7 signifies animal pollution, and a ratio between these two values indicates mixed origin (6). Geldreich has found, however, that this relationship is useless except when the fecal contamination is less than 24 hours old (6,7). Tables 5 and 6 substantiate the unreliability of the fecal coliform to fecal streptococcus ratio in the absence of any point source of pollution. At site #3, the ratio is almost always less than 0.7, even when there is evidence of human contamination (the high concentration of S. mitis and S. salivarius). On the other hand, it rises above 1.0 when there is no isolation of this biotype, during periods of low-flow conditions. Indeed, when the ratio approaches 4.0, there is evidence that most of the fecal streptococci are of vegetation origin, since there is a high proportion of S. faecium var. casseliflavus. At site #9 on June 29, when the ratio was greater than 1.0, indicating mixed human and animal pollution, there was no evidence of human pollution, but rather there was high concentration of animal fecal contamination as evidenced by the high concentration of S. bovis.

Discussion

In the study area, large slopes, shallow soils, large amounts of rainfall, and the presence of human and animal activity all combine to increase the impact of runoff pollution on surface waters beyond that experienced in a protected area of the watershed. Because the extent of this non-point pollution affects the surface water quality to the point where suggested water quality standards are violated, this impact is highly significant.

In 1965, Geldreich found a sharp increase in frequency of Salmonella detection when fecal coliform concentrations exceeded 200 per 100 ml of fresh water. He therefore suggests this level as a standard for recreational waters (5). Smith supported this suggestion by finding that in a river in a rural area, the geometric mean ratios relating pathogens to indicators were one Salmonella to 227 fecal coliforms and 367 fecal streptococci (26).

These levels of indicator bacteria were not exceeded in the protected watershed except during the peak of a hydrograph caused by heavy rainfall. However,the only time that these levels were not surpassed in the downstream portion of the creek was during winter months when steady state flow rate was much higher than during the rest of the year. This is particularly significant since most recreational use of surface waters takes place during the summer.

During storm events, the total biomass present in a stream as well as the actual concentration of bacteria deserves special consideration. An increase in bacterial load with lower bacterial

concentrations may be more detrimental to stream quality than lower water flow that has higher bacterial concentration and less total biomass (29). For example, if bacterial concentrations decrease by one half in the course of a storm event, but the flow rate is three times greater, the total biomass is increased. After bacteria enter a stream, their numbers decrease due to dilution, sedimentation, and die-off (21). The significance of increased loading is manifested in the settling and subsequent regrowth of enteric bacteria in sediment (10, 13). Runoff across land with unsatisfactory domestic drainfields can therefore have two undesirable impacts on water quality. The first is manifested immediately by the first flush phenomenon which carries high numbers of disease causing and fecal bacteria into surface waters. The second effect is manifested later and results from prior bacterial accumulation in sediments and their later shedding into water during warm, low flow conditions. This regrowth and shedding from sediments of enteric bacteria deposited during and following winter and spring storms probably accounts for the increased concentration of these bacteria during low flow summer periods in the unprotected regions of the creek.

In many previous studies of the sanitary significance of fecal streptococci, only enterococci have been considered (2, 12, 14, 15, 16, 17, 26). This emphasis is ironic, since these organisms have been isolated from 71% of mammals tested (7), 86% of reptiles (7), 32% of birds (7), 58% of insects (8), 70% of agricultural soils (14), and 28% of plants (8). Their ubiquity makes them unsuitable in defining their origin as fecal contamination.

On the other hand, there are several streptococcal organisms specific to given hosts. Streptococcus salivarius and S. mitis are human buccal streptococci which enter the digestive tract and populate feces in low numbers (23, 24, 25). At times, these organisms comprise the predominant streptococcal flora of the human intestine, but they are not invariably present (25). These organisms are considered sensitive indicators of human fecal pollution when encountered in the environment (4, 6, 11, 18).

Streptococcus bovis and S. equinus are found in significant numbers in cows, sheep, horses, pigs, ducks, dogs, and rodents. They are also present in trace amounts in chickens, turkeys, and cats (7). They are not found in human feces, nor are they encountered in the environment without concommitant fecal contamination from mammals other than humans (4, 6, 7).

The results of this study show that human activity has both direct and indirect effects on surface water quality deterioration. Increasing impervious areas in the form of rooftops and paved roads decreases the amount of precipitation which can be absorbed by the soils, so that runoff carrying bacterial loads is increased (22). Grazing domestic animals on slopes above the creek is certainly a factor in the stream pollution, as seen in the increase in S. bovis and S. equinus from site #9 to site #3 during runoff conditions.

However, human population is also directly responsible for contamination of the waters, as demonstrated by the high concentrations of the S. mitis and S. salivarius biotypes. This can be attributed to

the presence of septic drainfields in areas where, either because of a high water table, shallow soils, or large slopes, the soil is unsuitable for such use (27). This conclusion is further supported by the demonstration of Hagedorn et al (9) and Rahe et al (20) of the ability of bacteria to move long distances in a relatively short time in soils with gradients as low as 2%. They also found the rate of movement to peak during periods of heavy rainfall. This corresponds to the occasions when the human biotypes (S. mitis and S. salivarius) were isolated from areas downslope from the housing development.

When Geldreich first introduced the use of fecal coliform to fecal streptococcus ratios in the differentiation of sources of fecal contamination, he specified that they must be applied carefully (7). He found them most meaningful when developed from bacterial densities for samples taken at the point of discharge into a stream, or within a limited distance or flow time downstream. This implies that only a point source of pollution can be identified with this method. The results of this study uphold this concept. The ratios of these two indicator groups was found to be meaningless in evaluating runoff pollution. As seen in Tables 5 and 6, the higher ratios are more representative of low flow conditions than of human contamination.

However, the development of a biotyping scheme that can quickly identify strains of fecal streptococci is a promising technique for identification of fecal contamination sources. This study shows such a system to be both plausible and quite effective.

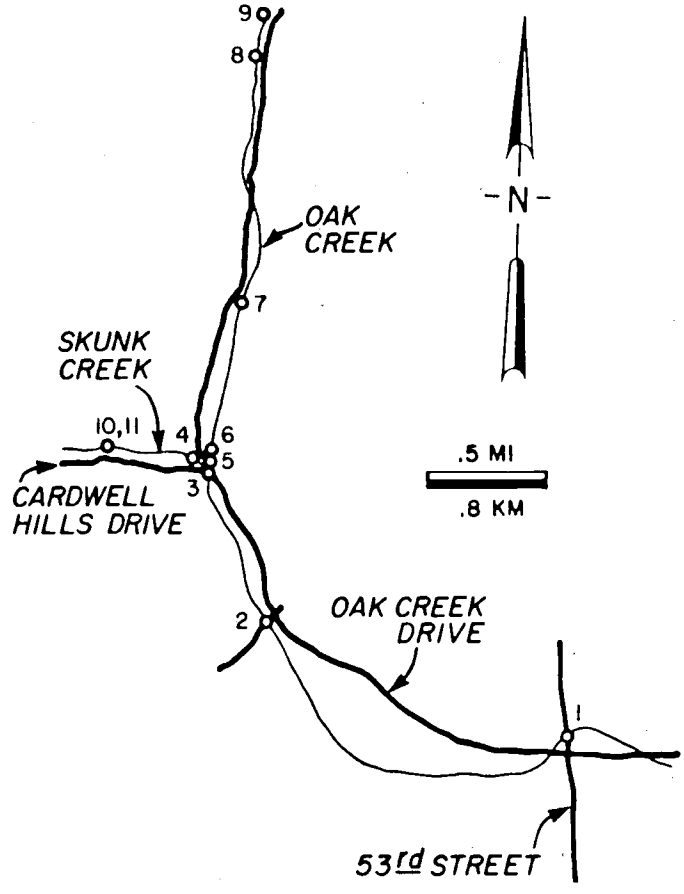

Fig. 1. Map of the study area, with sampling sites labelled.

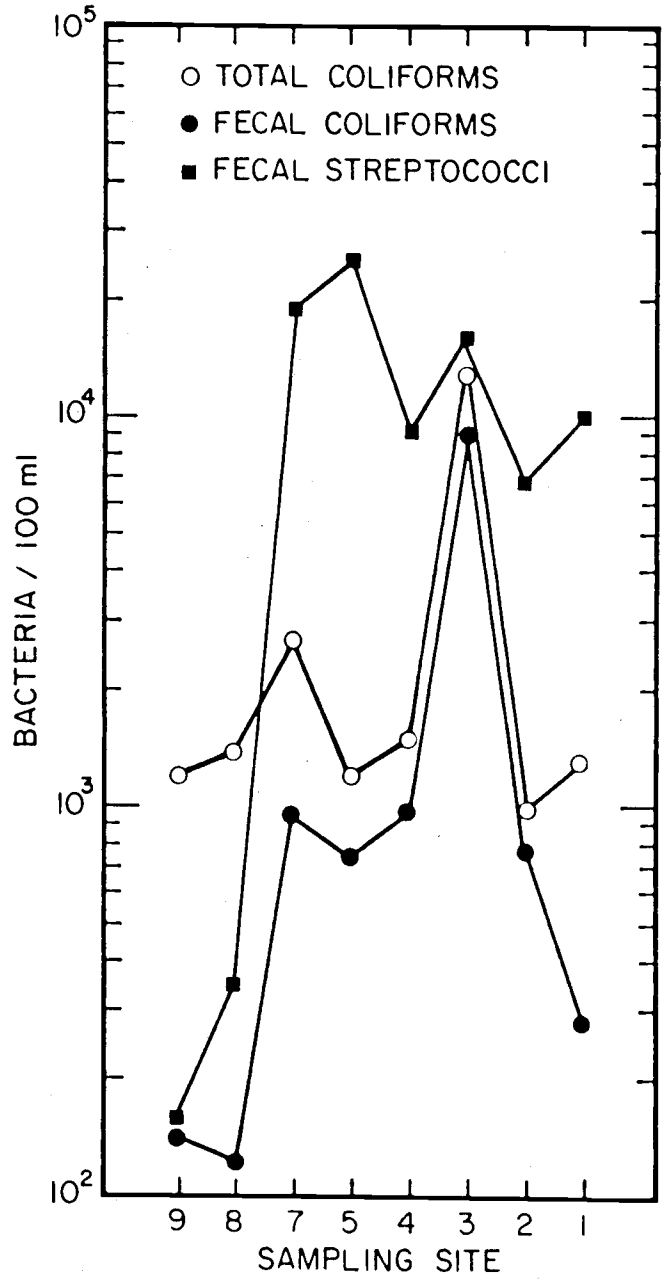

Fig. 2. A representative sampling of bacterial counts determined by culturing in duplicate, from upstream in a limited access forest (sites #8 and 9) and downstream through a rural development (sites #1-7).

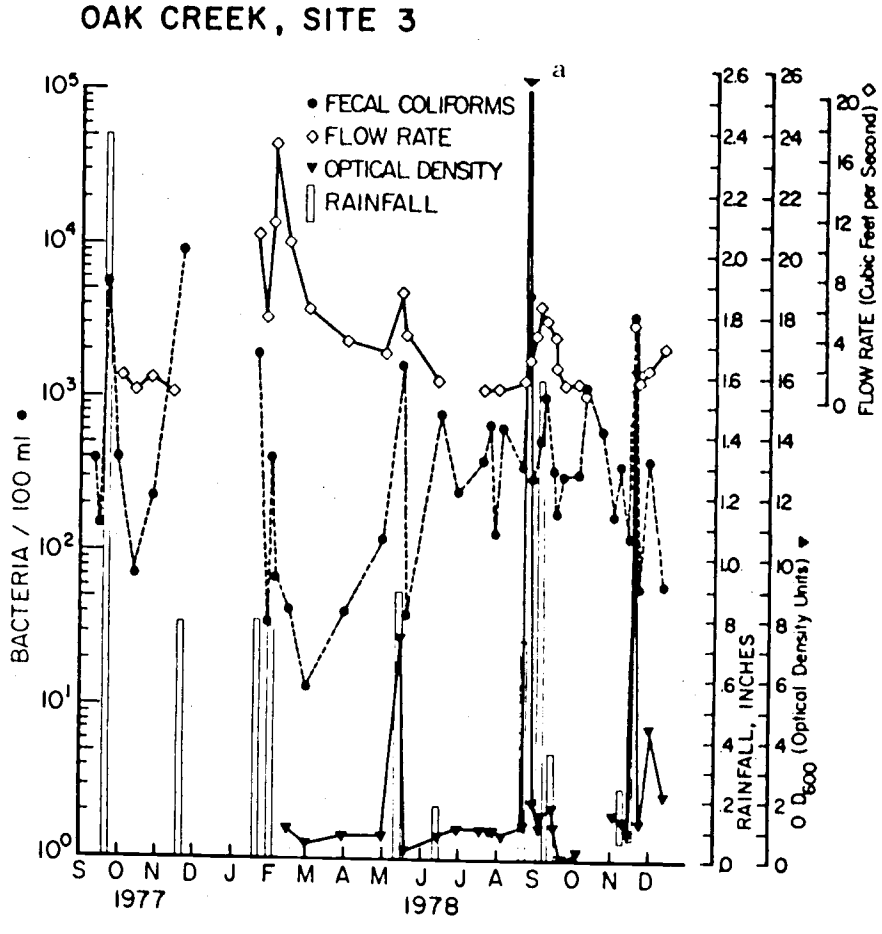

Fig. 3. Site #3: Fecal coliform levels, flow rate, optical density, and precipitation, from September, 1977, through December, 1978.

[a]optical density exceeds 26 OD units.

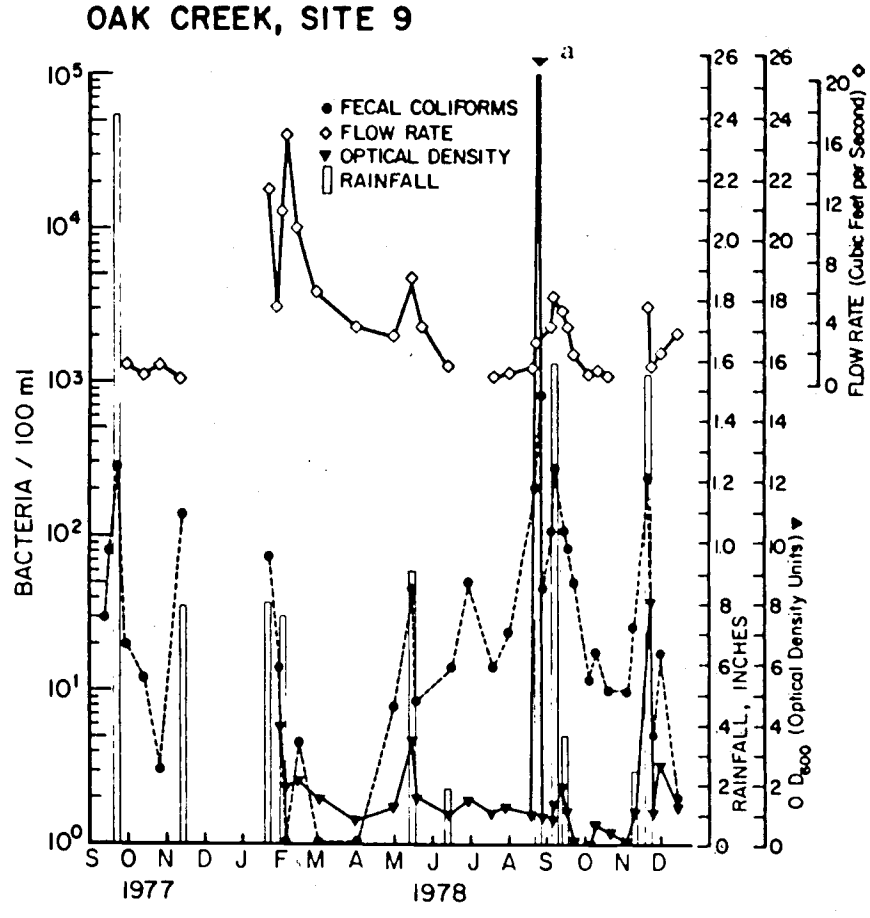

Fig. 4. Site #9: Fecal coliform levels, flow rate, optical density, and precipitation, from September, 1977, through December, 1978.

[a]optical density exceeds 26 OD units.

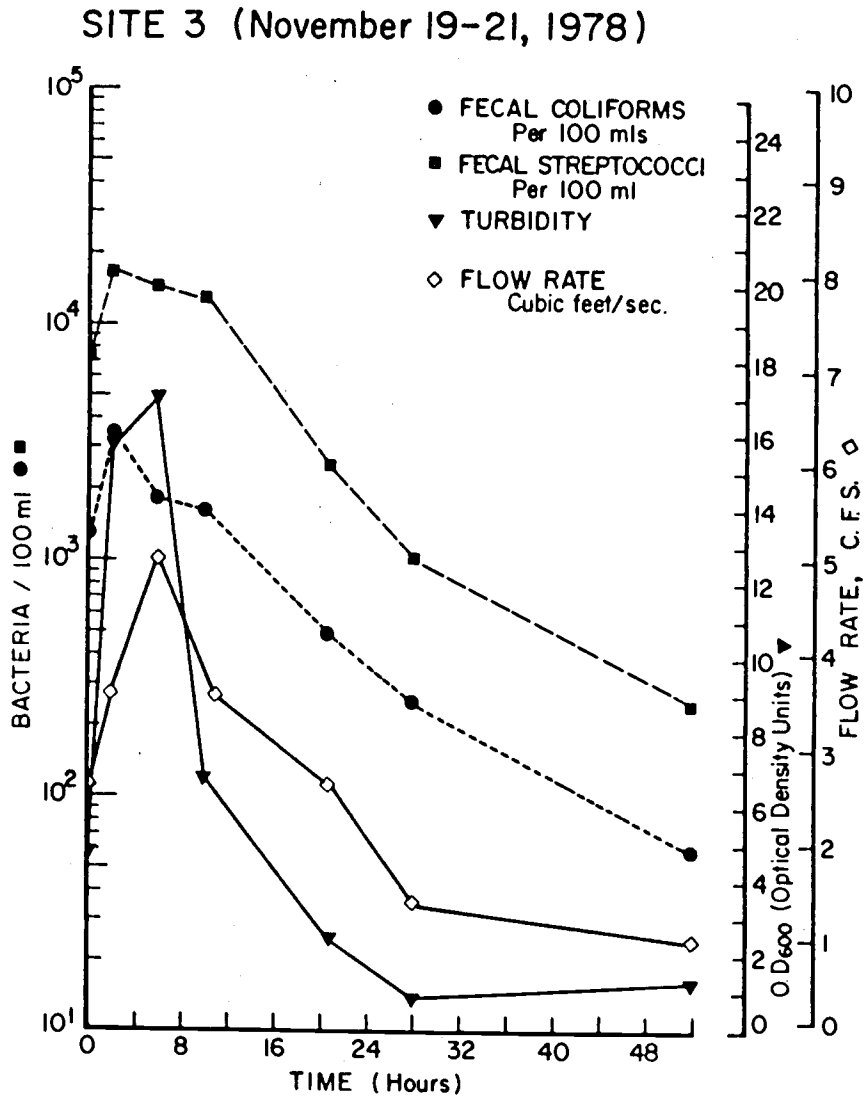

Fig. 5. Site #3: Fecal coliform and fecal streptococcus concentrations, flow rate, and turbidity during storm event of November 19, 1978.

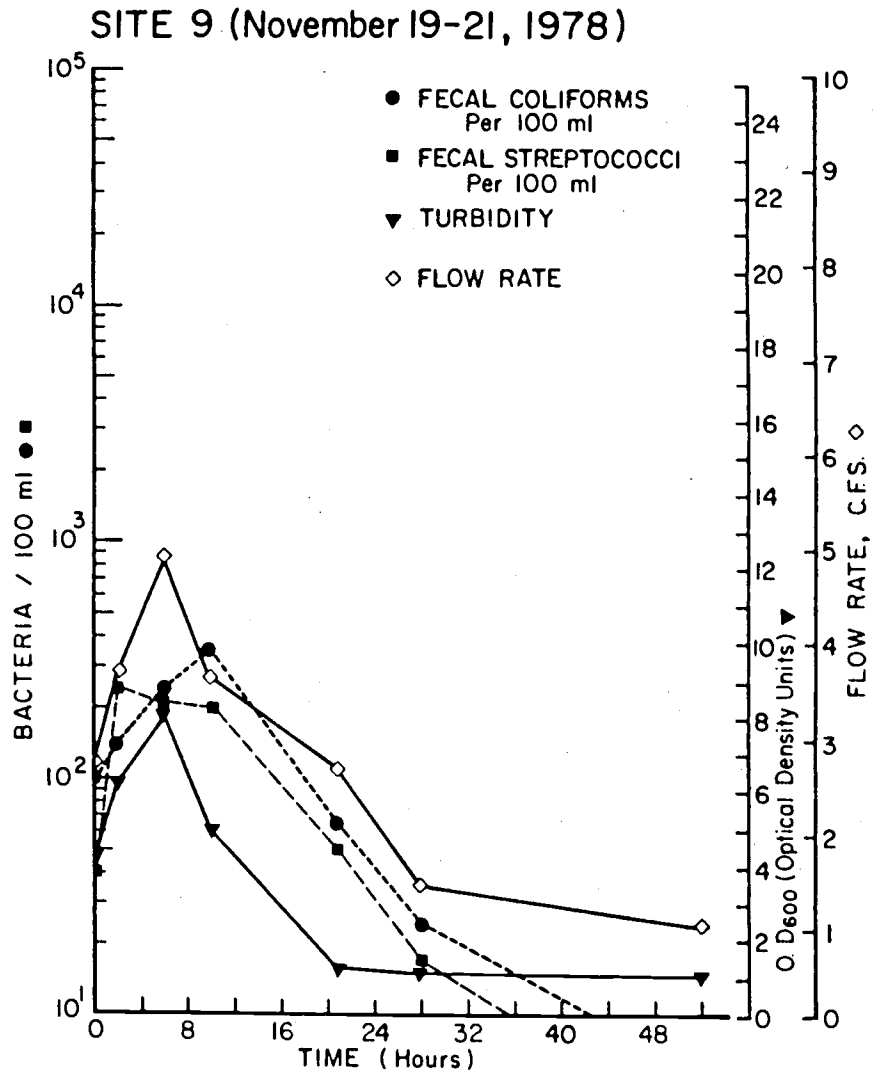

Fig. 6. Site #9: Fecal coliform and fecal streptococcus concentrations, flow rate, and turbidity during storm event of November 19, 1978.

Table 1. Biotype reactions and sources of fecal streptococci

Species	Reactions[a]						Source
	L	A	So	St	N	T	
S. faecalis	+	-	+	-	+	+	Human feces Soil Vegetation Mammalian feces
S. faecium	+	+	-	-	+	+	Human Feces Fowl feces Soil Vegetation Mammalian feces
S. faecium var. casseliflavus	+	+	+	-	+	+	Vegetation Soil Fowl feces
S. durans	+	-	-	-	+	+	Human feces Vegetation Soil Mammalian feces
S. bovis	+	±	-	+	-	-	Most non-human mammalian feces
S. equinus	-	-	-	-	-	-	Equine feces
S. mitis/ S. salivarius	+	-	-	-	-	-	Human feces

[a] L, lactose fermentation; A, arabinose fermentation; So, sorbitol fermentation; St, starch hydrolysis; N, growth on 6.5% NaCl agar (20); T, growth at 10C in BHI broth incubated for five days.

Table 2. Mean bacterial concentrations during steady state low flow and high flow, and at the peak of storm events

Site #9	TC/100 ml[a]	FC/100 ml[a]	FS/100 ml[a]	TPC/100 ml[a]
Low flow[b]	1.3×10^2	6.5×10^1	1.4×10^2	7.4×10^4
High flow[c]	1.2×10^2	6.0×10^0	1.9×10^1	9.5×10^3
Storm event[d]	1.4×10^3	2.7×10^2	7.0×10^2	2.1×10^5
Site #3				
Low flow[b]	8.7×10^2	3.9×10^2	1.1×10^3	2.5×10^5
High flow[c]	2.1×10^2	5.3×10^1	1.3×10^2	1.3×10^5
Storm event[d]	1.7×10^4	4.1×10^3	1.1×10^4	2.7×10^6

[a] TC, total coliforms; FC, fecal coliforms; FS, fecal streptococci; TPC, total plate count.

[b] Low flow values based on mean of four samplings.

[c] High flow values based on mean of three samplings.

[d] Storm event values based on peak values during first flush of storm event, mean of four samplings from four storm events.

Table 3. Correlation coefficients for storm event of Nov. 19-21, 1978

Site #3, (Oak Creek Road and Cardwell Hills Road)

	TC[a]	FS[a]	OD[a]	SRF[a]
FC	.32	.92	.87	.72
TC		.31	.23	.36
FS			.91	.87
OD				.88

Site #9, (West fork, Oak Creek)

	TC	FS	OD	SFR
FC	-.14	.80	.74	.80
TC		-.08	-.19	.16
FS			.89	.87
OD				.93

---

[a]FC, fecal coliforms; TC, total coliforms; FS, fecal streptococci; OD, optical density; SFR, stream flow rate

Table 4. Fecal streptococci isolated from raw sewage and Oak Creek water (site #3) on KF Streptococcus agar and PSE agar[a]

Species	Raw Sewage				Oak Creek, Site #3			
	KF		PSE		KF		PSE	
Enterococci	Number	Percent	Number	Percent	Number	Percent	Number	Percent
S. faecalis	35	70	23	48	17	36	10	21
S. faecium	12	24	13	27	3	6	6	12
S. faecium var. *casseliflavus*	1	2	4	8	20	43	8	17
S. durans	2	4	3	6	5	10	2	4
Non-enterococci								
S. bovis	0	0	0	0	1	2	10	21
S. mitis/ *S. salivarius*	0	0	5	11	1	2	12	25
Total	50	100	48	100	47	100	48	100

[a]One sample from each source, between 47 and 50 colonies randomly picked from each medium for biotyping

Table 5. Site #3: Fecal streptococci randomly isolated and biotyped, and fecal coliform to fecal streptococcus ratios, from May 14, 1978, through April 10, 1979

Date	No. of Strains Biotyped	Percent of strains identified as: S. faecium	S. faecium var. casseliflavus	S. faecalis	S. durans	S. bovis	S. equinus	S. mitis/S. salivarius	FC:FS[a] Ratio
May 14[b]	40	35	20	23	15	0	2	5	0.64
May 17	47	26	57	0	15	0	0	0	0.38
Jun 29	41	61	15	12	10	0	0	0	1.85
Jul 18	40	15	43	15	28	0	0	0	3.08
Sep 5[b]	45	0	40	11	7	4	0	36	0.10
Sep 13[b]	49	10	16	20	6	16	0	32	0.41
Oct 19[b]	49	4	8	8	6	0	0	73	0.34
Nov 29[b]	46	13	7	11	4	7	7	52	0.16
Jan 15[b]	47	17	13	21	13	0	4	32	0.53
Feb 16[b]	37	14	11	5	24	3	3	40	0.19
Mar 28	38	8	0	10	82	0	0	0	2.17
Apr 10[b]	40	15	22	48	10	0	0	5	0.88

[a]Ratios calculated from bacterial concentrations estimated on mFC agar and PSE agar, respectively.

[b]Time of sampling preceded by periods of rainfall and subsequent surface runoff.

Table 6. Site #9: Fecal streptococci randomly isolated and biotyped, and fecal coliform to fecal streptococcus ratios, from June 29, 1978, through April 10, 1979

Date	No. of Strains Biotyped	Percent of strains identified as: S. faecium	S. faecium var. casseliflavus	S. faecalis	S. durans	S. bovis	S. equinus	S. mitis/S. salivarius	FC:FS Ratio
Jun 29	46	6	9	46	6	33	0	0	1.09
Jul 18	19	0	32	58	10	0	0	0	0.11
Jul 31	50	0	18	68	14	0	0	0	0.36
Sep 5[b]	47	5	62	21	5	5	0	2	0.50
Oct 19[b]	9	0	11	22	67	0	0	0	--[c]
Nov 29[b]	13	8	23	23	38	8	0	0	--[c]
Jan 15[b]	5	0	0	40	60	0	0	0	--[c]
Feb 16[b]	26	0	23	50	27	0	0	0	--[c]
Mar 28	8	0	13	62	25	0	0	0	--[c]
Apr 10[b]	43	18	7	47	28	0	0	0	1.90

[a]Ratios calculated from bacterial concentrations estimated on mFC agar and PSE agar, respectively.

[b]Time of sampling preceded by periods of rainfall and subsequent surface runoff

[c]Fecal coliform and/or fecal streptococcus counts too low for accurate calculation of FC:FS ratios.

Literature Cited

1. American Public Health Association. 1976. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Inc., New York, New York.

2. Barnes, Ella M. 1964. Distribution and properties of serological types of S. faecium, S. durans and related strains. J. Appl. Bact. 27:461-470.

3. Facklam, Richard R. 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. 23:1131-1139.

4. Galvani, Mary Moriarty. 1974. Fecal contamination - the water analyst's responsibility. Part 1. Water and Sewage Works 121:66-69.

5. Geldreich, Edwin E. 1970. Applying bacteriological parameters to recreational water quality. J. Am. Water Works Assoc. 62:113-120.

6. Geldreich, Edwin E. 1976. Fecal coliform and fecal streptococcus density relationships in waste discharges and receiving waters. CRC Critical Reviews in Environ. Control 6:349-369.

7. Geldreich, Edwin E., and Bernard A. Kenner. 1969. Concepts of fecal streptococci in stream pollution. J. Water Pollut. Control Fed. 41:R336-R352.

8. Geldreich, E. E., B. A. Kenner, and P. W. Kabler. 1964. Occurrence of coliforms, fecal coliforms, and streptococci in vegetation and insects. Appl. Microbiol. 12:63-69.

9. Hagedorn, C., D. T. Hansen, and G. H. Simonson. 1978. Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow. J. Environ. Quality 7:55-59.

10. Hendricks, C. W., and S. M. Morrison. 1967. Multiplication and growth of selected enteric bacteria in clear mountain stream water. Water Res. 1:567-576.

11. Kenner, B. A., H. F. Clark, and P. W. Kabler. 1960. Fecal streptococci. II. Quantification of streptococci in feces. Am. J. Public Health. 50:1553-1559.

12. Kibbey, H. J., C. Hagedorn, and E. L. McCoy. 1978. Use of fecal streptococci as indicators of pollution in soil. Appl. Environ. Microbiol. 35:711-717.

13. Matson, Ernest A., Sally G. Hornor, and John D. Buck. 1978. Pollution indicators and other microorganisms in river sediment. J. Water Pollut. Control Fed. 50:13-19.

14. Mundt, J. O. 1961. Occurrence of enterococci: bud, blossom and soil studies. Appl. Microbiol. 9:541-544.

15. Mundt, J. Orvin. 1963. Occurrence of enterococci on plants in a wild environment. Appl. Microbiol. 11:141-144.

16. Mundt, J. O. 1973. Litmus milk reaction as a distinguishing feature between S. faecalis of human and non-human origins. J. Milk Food Technol. 36:364-367.

17. Mundt, J. O., W. F. Graham, and I. E. McCarty. 1967. Spherical lactic acid-producing bacteria of southern-grown raw and processed vegetables. Appl. Microbiol. 15:1303-1308.

17a. Neter, John and William Wasserman. 1974. Applied Linear Statistical Models. Richard D. Irwin, Inc., Homewood, Illinois.

18. Pavlova, Maria T., Francis T. Brezenski, and Warren Litsky. 1972. Evaluation of various media for isolation, enumeration and identification of fecal streptococci from natural sources. Health Lab. Sci. 9:289-298.

19. Qadri, S. M. Hussain, Charles W. Nichols, and S. G. M. Qadri. 1978. Rapid sodium chloride tolerance test for presumptive identification of enterococci. J. Clin. Microbiol. 7:238.

20. Rahe, T. M., C. Hagedorn, and E. L. McCoy. 1979. A comparison of fluorescein dye and antibiotic-resistant Escherichia coli as indicators of pollution in groundwater. Water, Air, and Soil Pollution. 2:93-103.

21. Reneau, R. B., Jr., J. H. Elder, D. E. Pettry, and C. W. Weston. 1975. Influence of soils on bacterial contamination of a watershed from septic sources. J. Environ. Qual. 4:249-252.

22. Rimer, Alan E., James A. Nissen, and David E. Reynolds. 1978. Characterization and impact of stormwater runoff from various land cover types. J. Water Pollut. Control Fed. 50:252-264.

23. Safford, C. E., J. M. Sherman, and H. M. Hodge. 1937. Streptococcus salivarius. J. Bact. 33:263-274.

24. Sherman, James M. 1937. The streptococci. Bact. Rev. 1:1-97.

25. Sherman, J. M., C. F. Nivin, Jr., and K. L. Smiley. 1943. Streptococcus salivarius and other non-hemolytic streptococci of the human throat. J. Bacteriol. 45:249-263.

26. Smith, R. J., R. M. Twedt, and Linda Krusel Flanigan. 1973. Relationships of indicator and pathogenic bacteria in stream waters. J. Water Pollut. Control Fed. 45:1736-1745.

27. United States Dept. of Agriculture, Soil Conservation Service. 1975. Soil survey of Benton County Area, Oregon.

28. Viraraghavan, T., and R. G. Warnock. 1976. Groundwater quality adjacent to a septic tank system. J. Am. Water Works Assoc. 68:611-614.

29. Wanielista, Martin P., Yousef A. Yousef, Waldron M. McLellon. 1977. Non-point source effects on water quality. J. Water Pollut. Control Fed. 49:441-451.

30. Winslow, C. E., and M. P. Hunnewell. 1902. Streptococci characteristics of sewage and sewage-polluted waters. Science 15:827.

Chapter 3.

The Effects of Seasonal Variation and Modern Zoning Criteria on Rural Drinking Water Supplies

Karla G. Lamka, Mark W. LeChevallier, and Ramon J. Seidler

Department of Microbiology, Oregon State University
Corvallis, Oregon 97331

Proofs, reprints, and other correspondence to:

Dr. Ramon J. Seidler
Department of Microbiology
Oregon State University
Corvallis, Oregon 97331

Technical Paper No. , Oregon Agricultural Experiment Station.

Abstract

On six occasions over a fifteen month period, the private well and spring water supplies in a modern rural neighborhood of 78 households were examined for total coliforms, fecal coliforms, Staphylococcus aureus, and standard plate count bacteria. More than one third of the water supplies were unsatisfactory on at least one occasion in terms of standard plate counts over 5×10^2/ml, the presence of coliforms, fecal coliforms, and/or Staphylococcus aureus. Citrobacter freundii, Klebsiella pneumoniae, and E. coli were the most frequently isolated total coliforms. At least 12 other genera of bacteria were identified from Standard Plate Count Agar. Coliform contamination was found to be higher following periods of rainfall, while high standard plate counts were more prevalent during warmer weather. These observations probably reflect leakage of surface water into improperly sealed wells or aquifer contamination during winter and the lack of chlorination to control microbial regrowth during the warm season. An inverse correlation was found between the presence of high standard plate counts and incidence of coliforms. Consumer education and a twice yearly monitoring of private water supplies appear necessary to reduce the incidence of water-borne disease organisms in private water supplies.

Introduction

Interest in the quality of drinking water supplies has been stimulated by the enactment of the Safe Drinking Water Act of 1974 and the dramatization of recent waterborne epidemics which occurred in recent years in the U.S. The goal of this Act is to improve the quality of drinking water supplies throughout the nation (16). To date, most of the research on this topic has been devoted to municipal supplies (11,17) since the population at risk from any treatment failure is much greater in public water supply systems than in smaller, private water supplies. However, 69% of the reported outbreaks have been from private supplies (8) which serve more than 30 million Americans (2). It has been estimated that consumption of untreated, contaminated groundwater, faulty well construction, and improper well location are the primary causes for these waterborne outbreaks (7).

In the present study an intensive bacteriological investigation was made on the incidence, numbers, and bacterial species present in the drinking water of private wells and springs which serve a modern rural community. Conventional indicator bacteria were not just monitored, but all coliforms present were identified to species. In addition, total plate count bacteria were isolated and identified. Although other rural drinking water supplies have been sampled (20,27), the present report is the first seasonal study of drinking water supplies conducted in a recently populated rural neighborhood, which was zoned by modern criteria.

Materials and Methods

Study area. A map of the study area is shown in Figure 1. The area consists of a recently zoned development covering approximately 1.3 square kilometers. It is located in the foothills of western Oregon, where the slope ranges from 3 to 50%.

The soil in the area belongs mainly to the Dixonville and Philomath series. The Dixonville series consists of well-drained, moderately deep soils of the fine, mixed, mesic family of Pachic Ultic Argixerolls. Dixonville soils are under-lain by weathered basalt bedrock at about 37 in. The Philomath series is a shallow, well-drained silty clay of the clayey, montmorillonitic, mesic, shallow family of Vertic Haploxerolls. These soils are underlain by a partly weathered basalt bedrock at a depth of about 18 in. (24).

There are about 78 households in the study area, most of which were built within the last five years. Lots are one to two acres in size, with single family dwellings. Water is obtained from groundwater supplies (private wells 80-200 ft. deep) or from surface springs. Waste is disposed of by septic tanks. There is a minimum distance requirement of 100 feet between septic tank and well.

Enumeration and identification techniques. Water samples were collected from each household, placed on ice, and processed within four hours.

Total and fecal coliforms were enumerated by the 15 tube most-probable-number technique through the completed step (3). All coliforms

from each contaminated sample were identified by the API 20E System (Analytab Produces, Inc., Plainview, N.Y.).

Standard plate count organisms were enumerated on Standard Plate Count Agar (Difco) after incubation at 35C for 48 h. Selected colonies of all morphological types were picked from Standard Plate Count Agar, and streaked for purification and maintained on slants of plate count agar. The cultures were identified according to Bergey's Manual of Determinative Bacteriology (5) and the scheme of Shayegani et al. (21). Ten percent of the gram negative cultures were confirmed by the API 20E System.

Standard plate count bacteria were identified using carbohydrate fermentation broth, tryptone broth, gelatin hydrolysis medium, standard plate count medium with 3 IU penicillin G, and oxidase test reagent (15). All were prepared according to standard procedures. Moeller arginine dihydrolase and lysine decarboxylase broths, phenylalanine agar, and oxidation-fermentation test media were prepared from BBL dehydrated media. Triple sugar iron agar slants, Simmons citrate slants, deoxyribonuclease (DNase) agar, and nitrate broth were prepared from Difco products. The inoculated media were incubated for up to five days at 35C.

Staphylococcus aureus was enumerated by membrane filtration through 0.45 μm porosity membrane filters (Gelman) which were placed onto Staphylococcus 110 medium (Difco) and incubated at 35C for 48 hours. Typical staphylococcal colonies were inoculated into Bacto-Coagulase Plasma (Difco) and the coagulase reaction was interpreted according to

the method of Sperber and Tatini (22). Coagulase positive bacteria were further identified as S. aureus by oxidase test (15), catalase test, gram stain and morphology. The ability of the bacteria to ferment glucose and mannitol anaerobically was determined using the media and procedures recommended by the Subcommittee on Taxonomy of Staphylococci and Micrococci (23).

Staphylococcal enterotoxins A, B, and C were obtained from Dr. Bergdoll, Food Research Institute, Madison, Wis. The antiserum was obtained from the Foods and Nutrition Department, Oregon State University. The enterotoxins were assayed by the microslide technique described by Casman and Bennett (6).

Results

During the course of the survey, 27 (35%) of the households were found to be using water contaminated with coliforms, fecal coliforms, Staphylococcus aureus or with standard plate counts exceeding 500/ml. Table 1 presents a summary of each sampling period, listing all incidences of total plate counts exceeding 500/ml and those samples which contained Staphylococcus aureus, coliforms or fecal coliforms. There was a seasonal trend in the quality of the water. For example, surveys conducted in October, 1977, January, 1978, and December 1978 were preceded within the previous 24 hours by periods of rainfall. During these periods, the highest incidence of coliform contamination was noted. The level of significance of the difference between coliform levels during periods of rain and no rain was $p = 0.10$. Another seasonally related parameter was the increased occurrence of high total plate counts during the warmer spring and summer months compared to the cool months of the year. This difference was significant at a level of $p = 0.05$.

The identity and incidence of isolates obtained from the completed tests for total and fecal coliforms, along with the number of samples which contained each species, are listed in Table 2. In those samples with total coliforms exceeding 2/100 ml, only two yielded just one species of coliform. The other ten samples yielded mixed cultures of at least two species of coliforms. Nine different species of enteric bacteria were identified during the study. E. coli comprised 13% of the total coliforms and 73% of the fecal coliforms. Citrobacter freundii

and Klebsiella pneumoniae, on the other hand, were the most commonly isolated total coliforms. On one occasion, Yersinia enterocolitica was isolated as a "typical gas producing coliform" (19). Fecal coliforms were isolated from two water supplies which used surface springs as the raw water source.

Table 3 presents the organisms isolated and identified from the standard plate count agar. There was a large variety of species isolated and a surprising number of these were opportunistic pathogens. It was, in fact, the presence of Staphylococcus aureus in the total plate counts that led to their being monitored in the subsequent samples. All of the Staphylococcus aureus isolates exhibited coagulase activity, and one half of them produced enterotoxin A.

Table 4 compares the bacterial plate counts and coliform incidence. It can be seen that incidence of coliform occurrence decreases as standard plate count exceeds 500/ml. Fecal coliform occurrence appears to be even more inhibited by high bacterial levels, since there is no occurrence of fecal coliform at standard plate counts exceeding 100/ml.

Discussion

In previous studies of rural drinking water supplies, as many as 90% of systems studied have been found to be contaminated with coliforms (20,27). However, many questions have remained unanswered. It has not been demonstrated to what degree modern zoning criteria would alleviate the problem of contaminated potable water supplies. Also needed is data concerning the effect of seasonal variation on such contamination. Finally, further emphasis on the threat posed by total plate count bacteria is necessary, regarding both coliform masking and the indication of the presence of nonfecal pathogens (11).

Total coliforms are the primary indicator organisms enumerated in investigations of drinking water supplies since they are indicative of surface runoff contamination. Their presence may also be significant in that they themselves may be opportunistically pathogenic (4,18), or capable of enterotoxin production (14). E. coli in drinking water represents the certain presence of recent fecal contamination (10), and possible bacterial pathogens, enteric viruses, or intestinal parasites. So it is significant that in 15% of the households the drinking water was contaminated with coliforms in spite of the up-to-date standards used in installing such wells.

Previous research has demonstrated the necessity of concurrent enumeration of standard plate count bacteria along with that of coliforms to properly evaluate the potability of a water supply (9,11). Because of their ability to mask coliform presence in both MPN and membrane

filtration techniques (9,11), neglecting to consider the total plate count could cause one to underestimate the health hazard of a given water supply. An example of this masking problem is reported in a study in Karachi, Pakistan: In 16 of 22 instances of pathogen occurrence in the absence of coliform detection, the standard plate count was greater than 500/ml (1). Other investigators have shown that Bacillus sp., pseudomonads, Flavobacterium sp., Actinomyces, and Micrococcus sp. can inhibit coliform growth (12,13,25,26). Table 3 shows that all of these antagonists were isolated in rural drinking water. Table 4 further demonstrates that high standard plate counts may also have been important in the assessment of the water supplies. As the total plate count rises from less than 10 bacteria per ml to between 11 and 500, the coliform incidence rises from two to eight percent. However, as the total bacterial population rises above 500/ml, the detection of coliforms drops to five percent. This supports a previous statement that standard plate counts greater than 500 organisms per ml adversely affects detection of coliform organisms (9,11).

In addition to their importance as coliform antagonists, many of the total plate count organisms isolated are pathogens, such as Staphylococcus aureus and Aeromonas hydrophila. Most of the other species have been implicated at one time or another as opportunistic pathogens (15). Because of the unexpected isolation of pathogens in the drinking water, the presence of Staphylococcus was investigated more thoroughly. Eight percent of the sampled households harbored Staphylococcus aureus, high levels of which were accompanied by excessive standard plate counts.

When Staphylococcus aureus reached 600/100 ml, the total plate count exceeded 1000/ml, while coliforms remained undetected. It thus appears that high standard plate counts may be useful as an indicator of the general drinking water quality and as an indicator for the presence of other pathogens or opportunistic pathogens when coliforms are not even detected.

This research also demonstrated the importance of education for the consumer who uses individual supplies for his drinking water. Much needs to be done to increase the awareness of the hazard of drinking contaminated water, and of ways of preventing contamination. Improper placement of wells, lack of sanitary seals, proximity of grazing animals to the well, and lack of knowledge of the significance of contaminated water were all found to be factors contributing to the poor quality water supply in this study.

More frequent monitoring was helpful in detecting contaminated water supplies which otherwise would have gone unsuspected, particularly during and following rainfall periods. Consumers need to know that testing water only upon installation of a well is an inadequate measure of potability of a water supply.

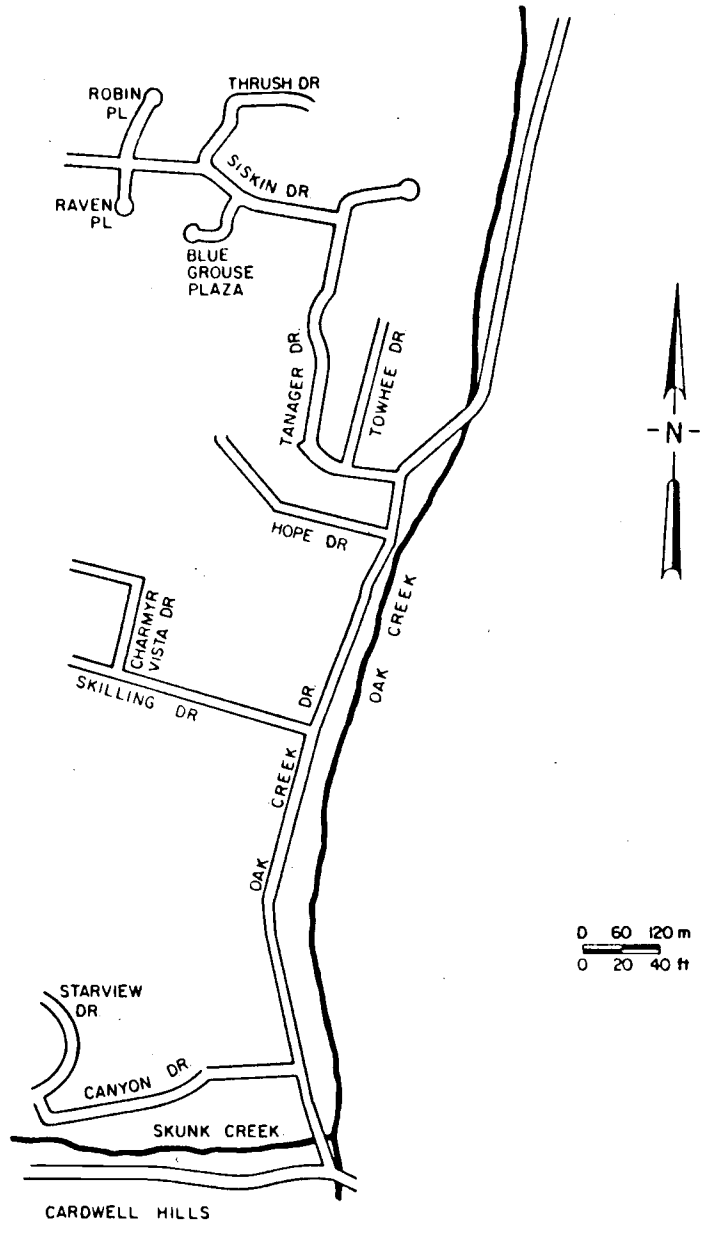

Fig. 1. Map of the study area where the water quality in approximately 78 homes was sampled in this study.

Table 1. Incidences of total coliform, fecal coliform, high standard plate count, and Staphylococcus aureus in rural drinking water supplies.[a]

Date	Sample Source	SPC[b] /ml	TC[b] /100 ml	FC[b] /100 ml	S. aureus /100 ml	Date	Sample Source	SPC /ml	TC /100 ml	FC /100 ml	S. aureus /100 ml
10/1/77	1	3.7×10^3	27	0	Not Tested	6/9/78	14	5.0×10^1	0	0	2
	15	5.7×10^3	0	0			25	1.9×10^3	0	0	0
	24	1.6×10^3	0	0			29	1.9×10^4	0	0	600
	30	1.1×10^1	34	0			36	3.0×10^1	2	0	0
	38	1.3×10^1	8	0			47	5.7×10^2	0	0	0
	39	1.2×10^2	8	0			50	3.1×10^1	13	0	0
	47	1.7×10^3	0	0			56	3.0×10^1	2	0	0
	48	3.1×10^1	5	0			63	7.2×10^2	2	0	0
	56	1.0×10^1	8	2			65	9.5×10^2	0	0	0
							73	5.7×10^2	0	0	0
1/4/78	30	7.7×10^3	0	0	Not Tested						
	48	1.0×10^1	2	0		10/4/78	39	1.5×10^2	2	0	Not Tested
	56	4.0×10^1	49	5			56	2.0×10^1	2	2	
	70	1.6×10^1	33	0			78	7.0×10^1	2	0	
3/28/78	17	1.0×10^2	0	0	1						
	40	5.1×10^3	0	0	0	12/29/78	30	5.4×10^2	0	0	Not Tested
	42	8.4×10^2	0	0	0		42	7.3×10^2	0	0	
	48	2.0×10^1	0	0	4		48	1.4×10^1	2	0	
	49	1.0×10^1	0	0	12		56	7.0×10^1	5	0	
	54	6.0×10^3	2	0	0		70	6.0×10^1	33	2	
	55	1.2×10^3	0	0	0		72	1.0×10^1	33	0	
	63	1.0×10^3	0	0	0						
	67	1.0×10^1	0	0	38						
	73	3.5×10^3	0	0	0						

[a] Samples are tabulated only for those specimens containing indicator organisms, S. aureus, and/or SPC in excess of 500/ml.

[b] SPC, standard plate count bacteria; TC, total coliforms; FC, fecal coliforms.

Table 2. Total and fecal coliform species isolated from rural drinking water supplies.

Identification	Number of Isolates	% of Total	Number of samples
	TOTAL COLIFORMS		
Citrobacter freundii	34	46	11
Enterobacter agglomerans	9	12	5
Enterobacter cloacae	3	4	3
Enterobacter hafniae	2	3	2
Escherichia coli	10	14	4
Klebsiella pneumoniae	13	18	6
Serratia liquefaciens	1	1	1
	FECAL COLIFORMS		
Citrobacter freundii	1	9	1
Escherichia coli	8	73	2
Serratia liquefaciens	2	18	1

Table 3. Bacteria identified from standard plate count agar in samples of rural drinking water.

Gram Positive	Gram Negative
Corynebacterium spp.	*Aeromonas hydrophila*
Arthrobacter spp.	*Pseudomonas acidovorans*
Actinomycetes	*Pseudomonas alcaligenes*
Bacillus	*Pseudomonas mallei*
Staphylococcus aureus	*Pseudonomas maltophilia*
Staphylococcus epidermidis	*Acinetobacter calcoaceticus*
Staphylococcus saprophyticus	*Alcaligenes denitrificans*
Micrococcus luteus	*Flavobacterium* spp.
Micrococcus roseus	*Moraxella bovis*
	Moraxella kingii
	CDC Group M-1
	CDC Group M-3
	CDC Group M-4
	CDC Group M-5
	Group IIk biotype 1
	Group IVe

Table 4. Standard plate count and the incidence of total and fecal coliform detection.

Standard Plate Count Density Range per 1 ml	Number of Samples	Total Coliform Occurrences	Total Coliform Incidence	Fecal Coliform Occurrences
≤10	61	1	1.6%	1
11-100	177	14	7.9%	3
101-500	52	4	7.7%	0
>500	19	1	5.2%	0

Literature Cited

1. Ahmed, Z., I. A. Poahni, and S. Siddiqui. 1967. Bacteriological examination of drinking water of Karachi and isolation of enteric pathogens. Pakistan Jour. Sci. Indust. Research 7:103-110.

2. Allen, M. J., and E. E. Geldreich. 1975. Bacteriological criteria for ground-water quality. J. Groundwater 13:45-52.

3. American Public Health Association. 1976. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Inc., New York, New York.

4. Bagley, S. T. and R. J. Seidler. 1978. Comparative pathogenicity of environmental and clinical Klebsiella. Health Lab Sci. 15:104-111.

5. Buchanan, R. E. and N. E. Gibbons (ed.). 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams and Wilkins Company, Baltimore, Maryland.

6. Casman, E. P., R. W. Bennett, A. E. Dorsey, and J. E. Stone. 1979. The microslide gel double diffusion test for the detection and assay of staphylococcal enterotoxins. Health Lab Sci. 6:186-198.

7. Craun, G. F. and L. J. McCabe. 1973. Review of the causes of waterborne-disease outbreaks. J. Am. Water Works Assoc. 65:74-83.

8. Craun, G. F., J. L. McCabe, and J. M. Hughes. 1976. Waterborne disease outbreaks in the U.S. -- 1971-1975. J. Am. Water Works Assoc. 68:420-424.

9. Geldreich, E. E. Is the total count necessary? Am. Water Works Assoc. first water quality technology conference, Dec. 2-4, 1973. Cincinnati, Ohio.

10. Geldreich, E. E., B. A. Kenner, and P. W. Kabler. 1964. Occurrence of coliforms, fecal coliforms, and fecal streptococci on vegetation and insects. Appl. Microbiol. 12:63-69.

11. Geldreich, E. E., H. D. Nash, D. J. Reasoner, and R. H. Taylor. 1972. The necessity of controlling bacterial populations in potable waters: community water supply. J. Am. Water Works Assoc. 64:596-602.

12. Hutchison, D., R. H. Weaver, and M. Scherago. 1943. The incidence and significance of microorganisms antagonistic to Escherichia coli in water. J. Bact. 45:29.

13. Kligler, L. J. 1919. Non-lactose-fermenting bacteria from polluted wells and sub-soil. J. Bact. 4:35-42.

14. Klipstein, F. A., L. V. Holdeman, J. J. Corcino, and W. E. C. Moore. 1973. Enterotoxigenic intestinal bacteria in tropical sprue. Ann. Intern. Med. 79:632-641.

15. Lennette, E. H., E. H. Spaulding, and J. P. Truant. 1974. Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.

16. Levin, Alan. 1978. The rural water survey. J. Am. Water Works Assoc. 70:446-452.

17. McCabe, L. J., J. M. Symons, R. D. Lee, and G. G. Robeck. 1970. Survey of community water supply systems. J. Am. Water Works Assoc. 62:670-687.

18. Martin, W. J., O. K. W. Yu, and J. A. Washington. 1971. Epidemiological significance of Klebsiella pneumoniae--a 3 month study. Mayo Clin. Proc. 46:785-793.

19. Nilehn, B. 1969. Studies on Yersinia enterocolitica with special reference to bacterial diagnosis and occurrence in human acute disease. Acta Pathol. Microbiol. Scand. Suppl. 206:1-48.

20. Sandhu, S. S., W. J. Warren, and P. Nelson. 1979. Magnitude of pollution indicator organisms in rural potable water. Appl. Environ. Microbiol. 37:744-749.

21. Shayegani, M., A. M. Lee, and L. M. Parsons. 1977. A scheme for identification of nonfermentative gram-negative bacteria. Health Lab Sci. 14:83-94.

22. Sperber, W. H., S. R. Tatini. 1975. Interpretation of the tube coagulase test for identification of Staphylococcus aureus. Appl. Microbiol. 29:502-505.

23. Subcommittee on taxonomy of staphylococci and micrococci recommendations. 1965. Int. Bull. Bacteriol. Nomencl. Taxon. 15:109-110.

24. United States Dept. of Agriculture, Soil Conservation Service. 1975. Soil survey of Benton County Area, Oregon.

25. Waksman, S. A. 1941. Antagonistic relations of microorganisms. Bact. Rev., 5:231-291.

26. Weaver, R. H. and T. Boiter. 1951. Antibiotic-producing species of Bacillus from well water. Trans. Kentucky Acad. Sci. 13:183-188.

27. Whitsell, W. T. and G. D. Hutchinson. 1973. Seven danger signals for individual water supply. Trans. Am. Soc. Agri. Eng. 16:777-781.