Comparative Survival of Indicator Bacteria and Enteric Pathogens in Well Water

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The comparative survival of various fecal indicator bacteria and enteric pathogens was studied in a stable well water supply by using membrane chambers. There was more variation in the 29 coliform cultures and they died more rapidly, as a group, than the 20 enterococcus cultures that were examined. The comparative survival of the organisms tested follows: *Aeromonas* sp. > the shigellae (*Shigella flexneri, S. sonneti*, and *S. dysenteriae*) > fecal streptococci > coliforms = some salmonellae (*Salmonella enteritidis* ser. paratyphi A and D, *S. enteritidis* ser. typhimurium) > *Streptococcus equinus* > *Vibrio cholerae* > *Salmonella typhi* > *Streptococcus bovis* > *Salmonella enteritidis* ser. paratyphi B. *S. bovis* had a more rapid die-off than did *S. equinus*, but both had significantly shorter half-lives than the other streptococci. The natural populations of indicator bacteria from human and elk fecal material declined similarly to the pure cultures tested, whereas the die-off of fecal streptococci exceeded the coliforms from bovine fecal material.

An understanding of the survival of fecal indicator organisms and the enteric pathogens in water is basic to the meaningful interpretation of sanitary water quality data. This is so because the isolation of fecal streptococci (12) or coliform bacteria (2, 7, 8, 16) is commonly used to signify the potential presence of intestinal pathogens. Although detection of indicator bacteria suggests occurrence of pathogenic organisms in water, the potential health hazard is dependant on retention of critical density levels and associated virulence for the pathogens in a given time frame during transmission via the water route. Furthermore, once these bacteria are deposited into the water they are in an environment that is not favorable to the maintenance of viability of most bacteria.

Studies have been performed to compare the survival of the fecal streptococci and the coliform bacteria (1, 9, 13, 14, 16). However, in each case either the bacterial populations were not defined or a highly artificial laboratory test system was used. Some authors (3, 5, 6, 9, 19) have also compared the persistence of a limited number of pathogens with an indicator culture in much the same way. From these studies, it was concluded that a few indicator bacteria survive somewhat longer than some enteric pathogenic bacteria. Therefore, experiments were needed in which the comparative persistence of a significant number and variety of indicator organisms and pathogens could be observed in a stable aquatic environment where the organisms could interact with a freely flowing water supply.

In the present investigation, separate membrane chambers (17) were filled with washed suspensions of fecal streptococci, coliform bacteria, mixed natural populations of indicator bacteria, or enteric pathogens. The chambers were then immersed in a flowing supply of well water, and the viability of each organism was evaluated with time. The results of these studies indicated that significant differences in the aquatic survival potential of these organisms exist. Also, the survival of the fecal coliforms and the fecal streptococci in the mixed natural populations from humans and elk declined similarly to the pure cultures tested, but the fecal streptococci from the bovine source declined faster than the coliform population. The implications of these findings are discussed in terms of the relative significance of the various indicator organisms in the aquatic environment.

**MATERIALS AND METHODS**

**Bacteria and media.** The indicator bacteria used in this study were isolated either from streams within the Bozeman Municipal Watershed or from the fecal
samples of various animals. Purified cultures of these coliform bacteria were identified by following the culture characteristics: growth in brilliant green lactose bile broth, characteristic colonies on eosin methylene blue agar, and the indole, methyl red, Voges-Proskauer, and citrate reactions (IMViC). According to the IMViC scheme, the coliform cultures were characterized as one of the following: Escherichia coli (++++), E. coli II (++ --), Enterobacter aerogenes (---+ +), Escherichia freundii (++ +), and such types as (+ + + +) and (+ + + +). Fecal coliform bacteria were determined by gas production from EC medium in 24 h at 44.5°C; both EC positive and negative types of these species were used.

The fecal streptococci used were characterized according to the following characteristics: growth in brain heart infusion broth within 2 days at 45°C and within 5 days at 10°C; NH₄⁺ production in peptone broth, gelatin liquefaction, growth on 0.02% potassium tellurite, mannitol fermentation, and hemolysis on heart infusion agar containing 5% horse blood. These cultures were identified as Streptococcus faecalis, S. faecium, S. liquefaciens, S. zymogenes, S. durans, and S. bovis. S. equinus was supplied by Don Reasoner, Environmental Protection Agency, Cincinnati. The Aeromonas species used was isolated from Lake Quinault, Washington State, and furnished by R. J. Seidler, Department of Microbiology, Oregon State University.

Cultures of pathogenic species used were obtained from a variety of sources. Vibrio cholerae 301 was furnished by Frank Newman, Veterinary Research Laboratory, Montana State University. Salmonella enteritidis ser. paratyphii A, S. enteritidis ser. typhimurium, and Shigella flexneri 2A were secured from the Center for Disease Control, Atlanta, Ga. Salmonella typhi, Shigella dysenteriae, Shigella sonnei, and Salmonella enteritidis ser. paratyphi B and D were obtained from the stock culture collection, Department of Microbiology, Montana State University.

All cultures used in these experiments except V. cholerae were grown for 24 h on Trypticase soy broth (BBL) with 0.3% yeast extract and 0.5% glucose added for 24 h at 37°C. V. cholerae was grown on brain heart infusion broth. Cells were harvested by centrifugation (3,020 x g) for 10 min and washed twice with either sterile buffer solution or autoclaved, unchlorinated well water. After the final wash, the cells were suspended in either the sterile buffer or the sterile well water and diluted to the desired population density with the same diluent. Except for V. cholerae, the bacteria were enumerated by following standard methods (2). V. cholerae was counted after being spread on heart infusion agar with sterile glass rods and incubated for 48 h at 37°C.

The mixed suspensions of bacteria were prepared for the survival studies from bovine and elk droppings and from raw domestic sewage. Fecal samples that were less than 4 h old were obtained from a herd of heifers that had not been given antibiotics and a herd of wild elk in early summer. The samples were then refrigerated. Eleven grams of pooled droppings from at least four animals were added to 100 ml of sterile well water and agitated for 1 min in a Sorvall Omni-Mixer at medium speed. The supernatants were decanted and diluted with well water to give an appropriate bacterial density. The raw sewage was settled for 2 h, decanted, and diluted. Each suspension was then used to fill separate, sterile membrane chambers. Standard membrane filter techniques (2) were used to enumerate these bacteria.

**Experimental apparatus.** The membrane chambers are described elsewhere (17). After the chambers were filled with bacterial suspensions, they were immersed in a 40-liter overflow tank (Fig. 1) filled with unchlorinated well water. Fresh well water was continually added to the tank at a rate of 5 liters per minute with mixing.

**RESULTS**

Previous studies of bacterial survival that were carried out by using membrane chambers filled with suspensions of indicator bacteria and immersed in streams (17) revealed that fluctuations in the water composition influenced the population dynamics observed. Because of this, comparative studies were unfeasible over a sustained period. Accidental leakage of pathogens from the chambers that might occur was another factor that prompted a search for a more satisfactory location for the study. An unchlorinated well at the Bozeman Municipal Wastewater Treatment Plant was used. Chambers were suspended in a tub through which the well water flowed at a measured rate. Overflow from the system entered the treatment facility. Water quality parameters (Table 1) were stable, and only minor variations in temperature (9.5 to 12.5°C) were observed during the year.

![Fig. 1. Photograph of the overflow tank used in the experiments. Membrane chambers loaded with bacterial suspensions were immersed in the water that was continuously exchanged.](image_url)
Aeromonas species, obtained from sewage. The raw human intestinal flora information observed. These 
cultures, amounts varied in the study. There was a lack of close agreement in survival pattern among 
coliform bacteria that were grouped together according to species, type, source, or characterization as 
fecal or nonfecal.

The survival of 22 representative fecal streptococcus cultures was also examined (Fig. 2). These 
cluded the seven species listed under Materials and Methods. The streptococci survived somewhat 
better as a group and exhibited less variation among cultures (standard deviation = 4.69 
bacteria/ml) than did the coliform bacteria (standard deviation = 8.48 bacteria/ml). Two exceptions to 
these observations were S. bovis and S. equinus. The die-off rates of these two cultures were greater 
than the other fecal streptococci and the coliform bacteria observed. An Aeromonas species exhibited 
a stable population in water as compared with the indicator bacteria examined (Fig. 2).

Survival of natural populations of bacteria. The survival of bacteria that were found in raw 
domestic sewage was studied to obtain information on the population dynamics of the human 
intestinal flora in water. The results (Fig. 3) indicate that the coliform bacteria and the fecal streptococci 
from this source followed die-off rates that generally agreed with the data obtained from pure cultures (Fig. 2). In 
addition, the fecal coliform population was consist-

TABLE 1. Some chemical characteristics of the well 
water used in the study

| Determination  | Value        |
|----------------|--------------|
| Conductivity   | 420.00       |
| pH             | 7.48         |
| Calcium        | 3.09         |
| Magnesium      | 0.97         |
| Sodium         | 0.38         |
| Potassium      | 0.10         |
| Sulfate        | 0.24         |
| Chloride       | 0.11         |
| Total cations  | 4.55         |
| Total anions   | 4.53         |
| Temperature    | 9.5 to 12.5 C|

* Micromhos at 25 C.
* Milliequivalents per liter.
* Temperature range for 12-month period of the study.

Survival of indicator bacteria. The survival of 51 cultures of indicator bacteria and an Aeromonas 
species was studied in the unchlorinated well water. A total of 29 coliform cultures, both fecal and nonfecal, isolated from 
water and from fecal samples of man and animals were used. Persistence of the various 
coliform types that were examined varied by the amounts indicated by the bars shown in Fig. 2. There 
was a lack of close agreement in survival pattern among coliform bacteria that were grouped together according to species, type, 
source, or characterization as fecal or nonfecal.

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addition, the fecal coliform population was consist-
ratio of fecal coliforms (FC) to fecal streptococci (FS) from 4.2 at zero time to 2.2 at day 4.

Similar experiments were done by using mixed natural populations from bovine and wild-elk fecal material. The resulting FC-FS ratios are seen in Fig. 3. The fecal coliform bacteria and the fecal streptococci from the elk declined at nearly the same rate, resulting in a slight increase in the FC-FS in 4 days (0.1 to 0.25). On the other hand, the fecal streptococci from the bovine source declined at a significantly higher rate than the fecal coliforms, resulting in an increase in the FC-FS from 0.25 at day 0 to 2.0 at day 4.

Survival of the pathogens. The survival of pure cultures of selected enteric pathogenic bacteria was studied in the same manner used for the indicator bacteria (Fig. 4). The persistence of several cultures (Shigella dysenteriae, S. sonnei, S. flexneri, S. enteritidis ser. paratyphi A and D, and S. enteritidis ser. typhimurium) followed similar patterns. The decline of viable S. typhi and V. cholerae was similar, but somewhat more rapid than that of the previous pathogen cultures. S. enteritidis ser. paratyphi B, however, died at a significantly higher rate than the other pathogens that were studied. The die-off rates of the individual pure cultures were determined and are listed in Table 2.

Influence of washing procedure on bacterial survival. Earlier survival experiments that were carried out indicated a more rapid die-off rate for many of the pure cultures under investigation. In these studies, where the cultures were washed with sterile phosphate buffer (2), all of the fecal streptococci and the pathogens (except S. sonnei) died nearly 10 times faster than when buffer with 0.2% gelatin or sterile well water was used. Because of this observation, and to more nearly approximate the natural situation, sterilized well water was used to wash all cultures.

DISCUSSION

The results of the experiments describing the survival of indicator bacteria in well water demonstrated that the fecal streptococci and the coliform bacteria remained viable to a similar extent under these experimental conditions. However, the viability of the coliform bacteria, as a group, declined slightly faster (half-time = 17 h) than the enterococci (half-time = 22 h). Similar results have been observed by others in an ice-covered Alaskan river (13), wastewater treatment lagoons (16), or bottles of natural waters (9, 14). Another worker (B. A. Benson, M. S. thesis, Univ. of Montana, Missoula, 1970), using bottles of water in which the pH and dissolved O2 were adjusted, obtained results indicating that coliforms persist somewhat longer than the enterococci in those environments. In these studies the manipulation of pH, aeration, and/or salt concentration might have caused the somewhat different results. This conclusion was also reached by Slanetz and Bartley (20) when they studied the population dynamics of these organisms in dialysis sacs that were immersed in sea water. Dialysis sacs filled with suspensions of bacteria were also used by Hendricks and Morrison when they observed some growth of selected indicator bacteria in a clear mountain stream (15). This observation was likely due to insufficient washing of the cultures or excessive populations of bacteria within the sacs. At no time in the present study was any growth observed in natural waters.

Fig. 4. Survival of water-borne enteric pathogens. Washed suspensions of bacteria were placed in membrane chambers that were immersed in well water. Samples were removed at intervals for bacterial enumeration. The cultures examined were S. dysenteriae, S. flexneri, S. sonnei, S. enteritidis ser. paratyphi A and D, and S. enteritidis ser. typhimurium (□), V. cholerae (■), S. typhi (●), and S. enteritidis ser. paratyphi B (■).
Table 2. Comparative die-off rates (half-time)\(^a\) of 
fecal indicator bacteria and enteric pathogens

| Bacteria                          | Half-time \(\text{h}\) | No. of strains analyzed |
|----------------------------------|------------------------|-------------------------|
| Indicator bacteria                |                        |                         |
| Coliform bacteria (avg)           | 17.0                   | 29                      |
| Enterococci (avg)                 | 22.0                   | 20                      |
| Coliform from raw sewage          | 17.5                   |                         |
| Streptococci from raw sewage      | 19.5                   |                         |
| Streptococcus equinus             | 10.0                   | 1                       |
| *S. bovis*                        | 4.3                    | 1                       |
| Pathogenic bacteria               |                        |                         |
| *Shigella dysenteriae*            | 22.4\(^b\)             | 1                       |
| *S. sonnei*                       | 24.5\(^b\)             | 1                       |
| *S. flexneri*                     | 26.8\(^b\)             | 1                       |
| *Salmonella enteritidis* ser. paratyphi A | 16.0\(^b\)  | 1                       |
| *S. enteritidis* ser. paratyphi D | 19.2\(^b\)             | 1                       |
| *S. enteritidis* ser. typhimurium | 16.0\(^b\)             | 1                       |
| *S. typhi*                        | 6.0                    | 2                       |
| *Vibrio cholerae*                 | 7.2                    | 3                       |
| *S. enteritidis* ser. paratyphi B | 2.4                    | 1                       |

\(^a\) The half-time was determined graphically from Fig. 2 and 4 as the time required for a 50% reduction in the initial population.

\(^b\) The half-time was determined graphically from Fig. 4 as the time required for a 50% reduction in the population at 24 h.

rapid die-off than a nonfecal variety at 20 C. However, this lack of close agreement in survival potential should not be surprising among bacteria that are as diverse as the coliforms.

As a group, the enterococci tested showed a higher degree of agreement among individual species with respect to survival and persisted longer than most of the coliforms tested. This might be related to salt concentration because some evidence has suggested a positive correlation between fecal streptococcus persistence in irrigation waters with the concentration of salts leached from the soil (E. E. Geldreich, personal communication). However, other studies (8, 12, 13) have also demonstrated the greater persistence of the enterococci as compared with the coliform bacteria. *Streptococcus bovis* and *S. equinus* were notably more susceptible to die-off in the aquatic environment. Others (9) have shown that *S. bovis* had a significantly higher die-off rate than other fecal streptococci and Geldreich (11) extended the observation to include *S. equinus*.

Mixed natural populations of indicator bacteria of human origin died in water very much as one might expect from the results of previous experiments. The die-off rates of the coliforms were slightly more rapid than the fecal streptococci (Fig. 3). The FC-FS decreased from over 4 at day 0 to around 2 after 4 days of exposure to the aqueous environment. The day 0 FC-FS value (4.2) is consistent with the observation by Geldreich (11) that a ratio greater than 4 indicates a human source, but the present study indicates that the ratio can decrease well below 4 during exposure of the bacteria to water. Therefore, these data show that this ratio in natural intestinal bacterial populations exposed to the aquatic environment diminishes to a point where it is no longer of significance in determining the source of the contamination when considering bacteria that originate from domestic sewage. This reinforces the previous conclusion (12) that the FC-FS is valid only during the 24 h immediately following the discharge of bacteria into the receiving stream.

Geldreich et al. (10) have observed that a FC-FS less than 0.7 usually indicates contamination from domesticated farm animals. This assertion was supported by the FC-FS of a mixed natural population from bovine fecal material that was 0.25 at the start of the experiment. However, this value increased to 2.0 after 4 days of exposure to water. In these studies the FC-FS of the bacteria from elk fecal material ranged from 0.1 at the start of the experiment to 0.3 after 4 days in the water. After this time, the FC-FS would likely remain less than 0.7 until virtual die-off occurred. The difference in the FC-FS versus time for the natural bacterial population from wild elk and domestic cows can be rationalized by the finding (our unpublished data) that bovine fecal material contains a much higher population of *S. bovis* (25% of fecal streptococci) than does elk (0 found in 100 streptococcus isolates tested). Therefore, because *S. bovis* has the highest die-off rate of the indicator bacteria tested, the FC-FS of the bacteria from this source would be expected to increase as a function of exposure time in water. On this basis, the FC-FS of the intestinal flora from a wild animal (elk) may be sufficiently stable in water to be useful in determining source, whereas this is not the case for bacteria from cattle. In practice, where one is usually unable to determine the specific animal that has contaminated a body of water in question, the FC-FS is of doubtful validity in identifying the source after the bacteria have been exposed to water for as short a time as 3 to 4 days.

From the initial survival experiments done in a water supply that was chemically and physi-
cally stable, useful information has been gained regarding the survival potential of indicator bacteria as compared with the enteric pathogens. Numerous workers have made similar studies (3, 5, 6, 9, 19) in both fresh and salt water. For example, great interest has been noted in the relationship between the survival of indicator bacteria and the salmonellae. Some have found that salmonellae survived better in water than the coliform bacteria (6) but most noted greater persistence of the indicator bacteria (3, 5, 9, 19). However, the results of these studies are difficult to compare due to the differences in the test systems and the organisms used. The results of the present study, which were obtained in a stable aquatic environment, indicate that the survival of both the coliforms and the fecal streptococci is on the same order as some of the salmonellae tested. In fact, _S. enteritidis_ ser. paratyphi A and D and _S. enteritidis_ ser. typhimurium as well as the shigellae tested (_S. dysenteriae, S. flexneri_ and _S. sonnei_) persisted in water slightly longer than some of the more stable coliforms and the streptococci. The concentration of inorganic constituents in the well water might favor the persistence of the shigellae as previously suggested (18). In contrast, _S. typhi_ had a much higher die-off rate (half-time = 6 h) that corresponded closely with _V. cholerae_ (half-time = 7.2 h) as observed by others (3). In addition, _Salmonella enteritidis_ ser. paratyphi B had the shortest half-time of the pathogens studied. It should be noted that the results suggest _Aeromonas_ species are of limited value as indicators of sanitary significance because of poor correlation with the survival of most pathogens in water.

The extrapolation of these comparative data, that were of necessity obtained under controlled experimental conditions, to the actual conditions in the field should be approached with great caution. This point was made some time ago by Batteau and Leurs (4). This is so because there are many variables in the natural aquatic environment such as water quality, phage, and competing or predatory organisms, any one of which may influence bacterial survival. Even so, some general conclusions can be drawn that could be of value in the interpretation of data in the field. Among these is the finding that there is wide variation in the persistence in both catagories of indicator organisms as well as the enteric pathogens. Because of this, the presence or absence of indicator bacteria in water might well be viewed with less significance under some circumstances. This is particularly true when considering the risk of the water-borne acquisi-

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