

## Bacteriological Quality of Runoff Water from Pastureland†

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Runoff from a cow-calf pasture in eastern Nebraska was monitored for total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS) during 1976, 1977, and 1978. Bacteriological counts in runoff from both grazed and ungrazed areas generally exceeded recommended water quality standards. The FC group was the best indicator group of the impact of grazing. Rainfall runoff from the grazed area contained 5 to 10 times more FC than runoff from the fenced, ungrazed area. There was little difference in TC counts between the two areas, but FS counts were higher in runoff from the ungrazed area and reflected the contributions from wildlife. Recommended bacteriological water quality standards, developed for point source inputs, may be inappropriate for characterizing nonpoint source pollution from pasture runoff. The FC/FS ratio in pasture runoff was useful in identifying the relative contributions of cattle and wildlife. Ratios below 0.05 were indicative of wildlife sources and ratios above 0.1 were characteristic of grazing cattle. Occasions when the FC/FS ratio of diluted cattle waste exceeded one resulted from differential aftergrowth and die-off between FC and FS. The FC/FS ratio and percentage of *Streptococcus bovis* in pasture runoff are useful indicators for evaluating the effectiveness of livestock management practices for minimizing bacterial contamination of surface water. The importance of choice of medium for the enumeration of FS in runoff derived from cattle wastes is discussed.

In the past, water pollution control policies have concentrated on abatement of municipal and industrial point sources, while the relative contribution from nonpoint sources was assumed to be small. More recently, the validity of this assumption has been seriously questioned. An estimated one-third of the pollutants entering United States waters comes from nonpoint sources. Current water quality legislation (P.L. 92-500, Sec. 208) requires the evaluation of nonpoint source pollutants and the implementation of plans to abate such pollutant discharge into navigable waters in the United States by 1985.

The movement of animal wastes into surface and ground waters is often cited as a major factor contributing to the pollution of available water in many regions. Over one-third ( $300 \times 10^6$  ha) of the land area of the continental United States is used for grazing livestock and receives 50% of all livestock wastes (U.S. Department of Agriculture, Agricultural Statistics, 1977). Consequently, the evaluation of the impact of animal-grazing operations on water quality is an important component in assessment and imple-

mentation plans for abatement of pollution from nonpoint sources. Information available on the impact of cattle-grazing operations on water quality indicates that pollution problems are usually associated with increased sediment or bacterial counts in runoff water (17, 19). The bacteriological quality of the runoff water is evaluated by comparison with recommended water quality standards. At best, the use of such standards, usually developed for evaluation of point source inputs, is tenuous because nonpoint runoff from agricultural cropland, ungrazed pasture, and urban areas often exceeds the same standards (10, 24, 25).

The impact of cattle grazing on the bacteriological quality of local or regional waters can only be evaluated if the parameters used to monitor water quality are singularly characteristic of the nonpoint source. The measurement of fecal coliforms (FC) is reported as the most reliable indicator of the fecal pollution of water, but this parameter does not identify the source (10, 12, 18). Analysis of water for both FC and fecal streptococci (FS) has been suggested as a method for determining whether fecal contaminants are from human or other animal sources (8). Water with an FC/FS ratio greater than 4 usually indicates pollution from domestic waste

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water; and when the FC/FS ratio is less than 0.7, contamination from nonhuman animal wastes is indicated (7). Identification of the streptococcal species present in water has also been suggested as a means of identifying sources of contamination. The predominant microflora of cattle feces is the short-lived starch hydrolyzer, *Streptococcus bovis*. The presence of *S. bovis*, as the predominant species in polluted water, implicates farm animals as the contaminating source (8, 16, 21).

A main purpose of the study reported here was to determine the bacteriological characteristics of pasture runoff and to compare them with runoff from an ungrazed area. We chose FC and FS as the major bacterial indicators of water quality, with hopes that the FC/FS ratios could be used to identify the source of the fecal "pollutants" in runoff water. The percentage of starch-hydrolyzing FS in runoff water was evaluated as a specific indicator of the inputs of grazing cattle.

#### MATERIALS AND METHODS

**Site description.** The experimental pasture containing the contributing watershed of this study (Fig. 1) is located at the U.S. Meat Animal Research Center at Clay Center, Neb. The average annual precipitation in this area is 64 cm (25 inches), and the pasture grass is predominantly bromegrass (*Bromus inermis* Leyss). The fenced pasture area is 40 ha (100 acres) in size and is ordinarily grazed by 35 to 45 cow-calf pairs. A small area (0.11 ha) immediately adjacent to the study watershed, from which livestock were excluded, was used to establish background levels for runoff water quality under ungrazed conditions. A discrete runoff area was obtained by use of an aluminum border. This area will be referred to hereafter as the ungrazed area. Runoff from grazed and ungrazed areas was directed through H flumes capable of measuring peak discharge. Total runoff was determined with water stage recorders on each flume. Rainfall was measured by using a standard U.S. Weather Bureau gauge.

**Bacterial analyses.** Surface runoff samples for bacteriological analyses were collected, by hand, from flume discharges in sterile 1-liter polypropylene bottles. Whenever possible, samples were collected at intervals which represented early, middle, and late segments of each runoff event. Samples were stored at 4 to 5°C until laboratory analyses could be completed. All samples were processed and incubated within 24 h after sampling.

The bacterial counting procedures for total coliforms (TC), FC, and FS were standard methods, as outlined by the American Public Health Association (1). The multiple-tube lactose fermentation test was used as a presumptive test for TC. FC were determined by the membrane filter technique, using 0.45- $\mu$ m membrane filters (Millipore Corp.) and M-FC medium (Baltimore Biological Laboratory). FS were determined by pour-plate counts, using Pfizer selective enterococcus (PSE) agar.

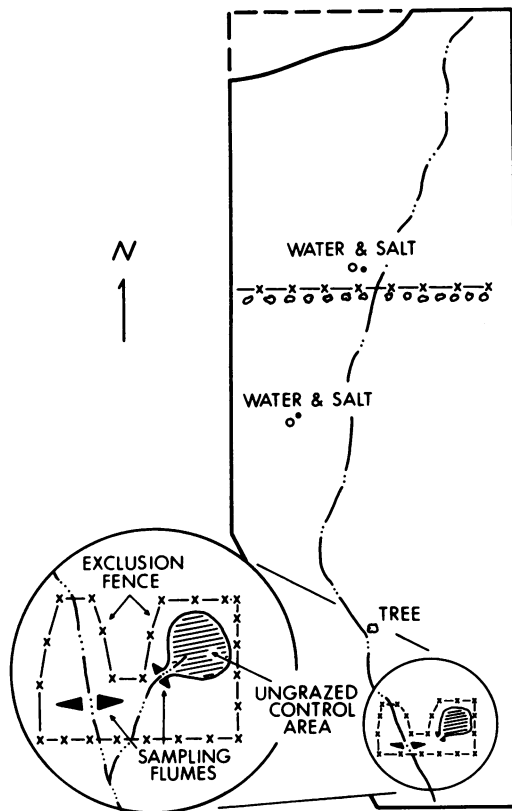


FIG. 1. Pasture site, illustrating watering areas in the upper pasture and sampling points at the south end.

Starch-hydrolyzing FS were enumerated by using the membrane filter technique with a starch-containing PSE agar overlayer (E. D. Oliver, M.S. thesis, South Dakota State University, Brookings, 1974). After the sample was filtered, the membrane filter was placed in a Petri plate (60 by 25 mm) and was overlaid with 5 ml of PSE agar containing 0.2% amylose azure (Calbiochem) and 0.01% 2,3,5-triphenyltetrazolium chloride. After incubation at 35°C  $\pm$  0.5 for 48 h, characteristic red colonies were counted as FS. Starch-hydrolyzing FS, presumably *S. bovis*, were counted at 12 to 16 h, 24 h, and totaled at 48 h. These times were chosen to allow better visualization of hydrolysis zones. Starch hydrolysis was evidenced by the formation of clearing zones around red colonies against a blue background.

**Laboratory incubations.** A study was conducted to determine the population changes of FC, FS, and starch-hydrolyzing FS with time in autoclaved runoff water that was inoculated with cattle feces and stored at 4 and 21°C. The inoculum was a composite (six subsamples) of manure that had been freshly deposited by cattle grazing on pasture. The composite sample was transported at 37°C and 3 h after sampling was diluted 10<sup>-2</sup> to 10<sup>-3</sup> with autoclaved runoff water in 150-ml milk dilution bottles and incubated at 4 and

21°C. These dilutions were chosen to simulate bacterial concentrations which had been found in pasture runoff. Each dilution and temperature series was replicated three times. Bacterial counts were made every 8 h for 2 days.

**RESULTS**

**Bacteriological quality of snowmelt runoff.** During the 3-year study, there were 10 snowmelt runoff events—two in 1976 and 8 in 1978. The data for indicator counts of snowmelt runoff (Table 1) represent the range for averages of one to two samples per event. The levels of TC in snowmelt runoff from both grazed and ungrazed pasture areas (Table 1) exceeded recommended water quality standards (Table 2). FC counts, often considered a better index of fecal contamination, were within recommended standards. Since snowmelt runoff occurred in early spring before animals were placed on the pasture, the FC counts in runoff from the grazed pasture were minimal.

The increase in FS counts in the ungrazed as compared with the grazed area was also reflected by a much lower FC/FS ratio in runoff from the ungrazed area. While sampling, we observed an increase in wildlife activity in the smaller, better protected, control area—e.g., numerous fecal droppings from field mice and rabbits.

**Rainfall runoff.** Because of dry weather conditions, only two rainfall runoff events occurred during the 1976 season. In contrast, the 1977 season was very wet (108 cm of rain), and 20

events were sampled for bacteriological analyses from the grazed pasture area. All 1977 data are expressed as averages of from two to three samples for each event. Bacterial counts were generally highest with the early peak runoff flows and decreased with time thereafter.

Counts for TC and FC (Fig. 2a and b) from both grazed and ungrazed pasture areas usually exceeded recommended water-quality standards. Counts for TC and FC were within recommended standards only when temperatures were low (both areas) or when cattle were absent from the main pasture. There was little difference between TC counts for the two areas, but FC counts were 5 to 10 times higher in runoff

TABLE 1. Bacteriological water quality indicators in snowmelt runoff from pasture for 1976 and 1978

Indicator	Runoff characteristics	
	Grazed area	Ungrazed area
TC <sup>a</sup>	7,900-330,000	7,000-1,700,000
FC <sup>a</sup>	0-110	0-220
FS <sup>a</sup>	0-6,000	7,500-162,000
FC/FS ratio	<0.01-0.06	<0.001-0.002

<sup>a</sup> Bacterial counts expressed as organisms per 100 ml. Median values for ranges given were very similar to computed averages of same data.

TABLE 2. Bacteriological water quality standards<sup>a</sup> (surface waters)

Water use	TC	FC
Recreation		
Primary contact	1,000	200
Partial contact	5,000	1,000
Public water supply	10,000	2,000

<sup>a</sup> U.S. Department of Interior, Federal Water Pollution Control Administration. A report of the Committee on Water Quality Criteria. Washington, D.C. 1968. Counts are expressed as organisms per 100 ml.

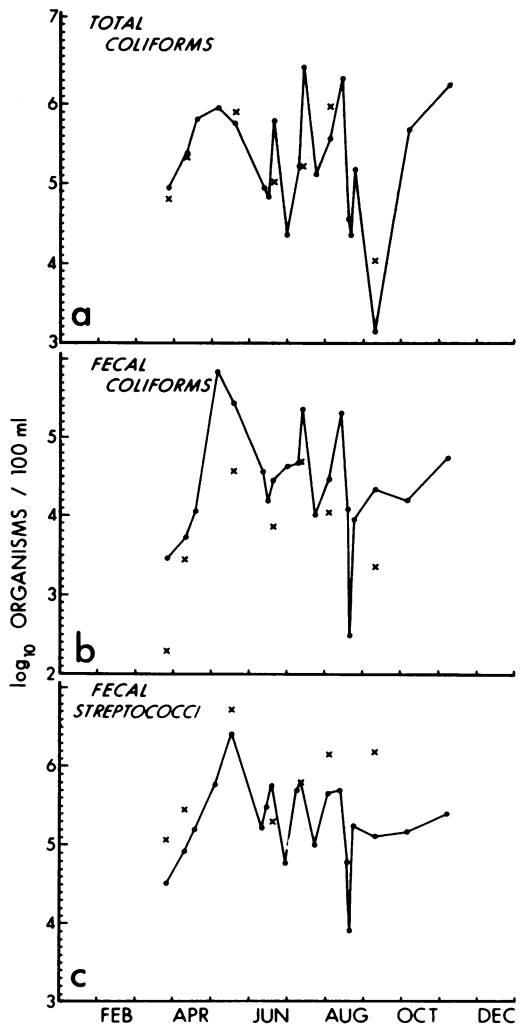


FIG. 2. TC (a), FC (b), and FS (c) counts in 1977 rainfall runoff from grazed (●) and ungrazed (×) pasture areas. Values expressed are averages for each runoff event.

from the grazed area as compared with runoff from the ungrazed area.

FS counts in rainfall runoff from the ungrazed area were consistently higher than counts in runoff from the grazed pasture (Fig. 2c). These high FS counts in the ungrazed area are best explained, as stated earlier, by the increased wildlife activity noted in this area.

We found no relationship between FC and FS counts in rainfall runoff (Fig. 2b and c) and either rainfall or total runoff for most events. However, there was an apparent relationship between FC and FS counts in runoff from the main pasture and animal stocking density and air temperature (Fig. 3), but neither statistic alone could account for all variations throughout the season. The decrease in air temperature noted on 22 August 1977, when cattle were off the pasture, was paralleled by minimum counts for FC and FS for this date. Air temperature was used for comparisons, instead of water temperature, because data were available for all events, and there was little or no difference between air and water temperature for rainfall runoff.

A comparison of FC/FS ratios in runoff between the grazed and ungrazed pasture areas revealed the differences in FC and FS levels in runoff from each area (Fig. 4). The FC/FS ratio in runoff from the grazed pasture ranged from 0.04 to 1.2 with an average of 0.26 for the season. The FC/FS ratios were lowest during times of the year when cattle were off the pasture or present in very low numbers (Fig. 3). The FC/FS ratio in runoff from the ungrazed area was much lower than that from the grazed area, because of lower FC and higher FS counts, and averaged 0.023 with a range of 0.001 to 0.08.

**Die-off and aftergrowth relationships.** The effects of cattle management on bacterio-

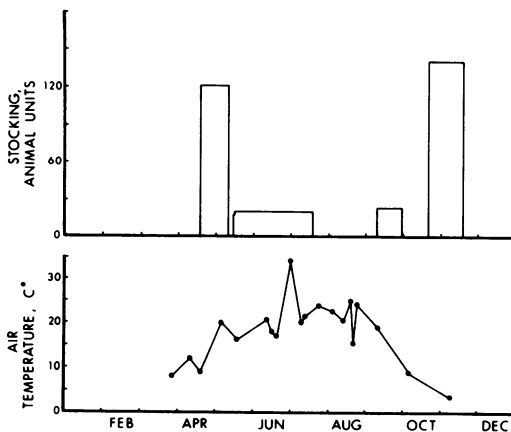


FIG. 3. Animal stocking rate and air temperature for 1977.

logical characteristics of runoff are shown in Table 3. In 1976 the weather was very dry for the 3 months before the 26 September rainfall event. The cattle (299 animal units), which had been placed on the pasture on 24 September, had remained in the upper pasture near the watering areas because of the dry conditions and the less succulent pasture forage. The FC/FS ratio in runoff at this time was only 0.014, with no starch hydrolyzers detected. Analysis of runoff 1 week later (3 October), when cattle were uniformly distributed across the pasture, revealed the presence of starch-hydrolyzing FS and an average FC/FS ratio of 3.0. The increase in both indicators apparently resulted from the presence of fresh feces in the October runoff, since animals approached within 10 m of the

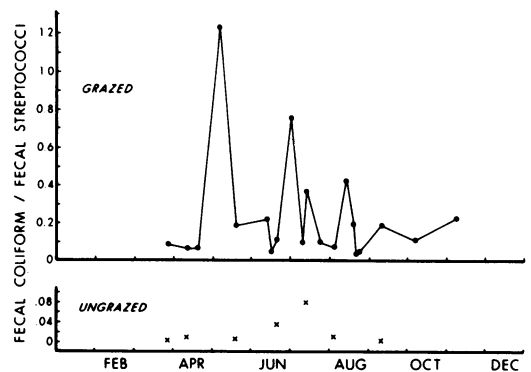


FIG. 4. FC/FS ratios in 1977 rainfall runoff from grazed (●) and ungrazed (×) areas. Values expressed are averages for each runoff event.

TABLE 3. Bacteriological water quality indicators in pasture runoff as related to cattle grazing distribution

Bacteriological indicator	Grazing distribution at time of runoff <sup>a</sup>	
	Localized <sup>b</sup>	Uniform <sup>c</sup>
FC	14,000 <sup>d</sup>	1,100,000 <sup>d</sup>
FS	1,000,000 <sup>d</sup>	380,000 <sup>d</sup>
Starch-hydrolyzing FS	0 <sup>e</sup>	22 <sup>e</sup>
FC/FS ratio	0.014	3.0

<sup>a</sup> 26 September for localized grazing and 3 October for uniform distribution.

<sup>b</sup> Cattle (299 animal units) were placed on pasture 24 September 1976. Rainfall ended a drought period so the grass was dry at time of runoff, and animals were congregated at northwest end of pasture around watering areas.

<sup>c</sup> Grass was more succulent and palatable as a result of rainfall, so the cattle were uniformly distributed across the pasture when the runoff occurred.

<sup>d</sup> Organisms per 100 ml.

<sup>e</sup> Percentages.

main sampling flume during the runoff event. The area immediately above the main flume was not fenced off until late October 1976.

The relationships between animal proximity to the sampling site and starch-hydrolyzing FS and FC/FS characteristics of runoff led us to believe that aftergrowth and/or die-off of FC and FS might be occurring. To test this hypothesis, we initiated a laboratory study to evaluate the influence of time and incubation temperature on the population dynamics of FC, FS, and starch hydrolyzers in autoclaved runoff water. FC or FS counts for samples incubated at 4°C changed little (Table 4). The percentage of starch-hydrolyzing FS tended to decrease with time. At 21°C, the FC counts in runoff water increased over 300-fold within 48 h. The increased FC/FS ratio with time was the result of the increase in FC counts. Although the total FS count changed little with time, the percentage of starch-hydrolyzing FS (presumably *S. bovis*) decreased from an initial maximum of 81% to minima at 40 h of 19 and 1% at 4 and 21°C, respectively. This indicated that populations of non-starch-hydrolyzing FS had increased during the incubation.

Applying data from our laboratory study for incubation times greater than 8 h to field conditions would be inappropriate because the maximum lapse time for runoff over the total length of pasture was less than 4 h.

### DISCUSSION

The bacteriological quality of runoff from pasture and rangeland often exceeds water quality standards. In our study, TC counts in runoff from both grazed and ungrazed pasture exceeded

recommended water quality standards (Table 2) over 90 and 98% of the time for use in primary body contact recreation and drinking-water supplies, respectively. However, as already discussed, we used the "presumptive" test for TC, and all State and Federal standards involving the most probable number of TC measurements now require use of the "confirmed" most probable number test for TC as a minimum form of analysis. FC counts were a better index of the fecal contributions of grazing cattle. Even so, 95% of the rainfall runoff samples from the ungrazed control area exceeded the recommended standard (200 FC/100 ml) for primary contact recreation. These results agreed with the findings of Robbins et al. (19), who reported yearly mean FC counts in runoff from grazed and ungrazed watersheds in North Carolina of 30,000 and 10,000 organisms per 100 ml, respectively.

Runoff from lands devoid of human activity or domestic animals contains bacterial indicator organisms. Doty and Hookano (5), who analyzed stream water from three pristine watersheds in northern Utah that had been protected from fire, domestic livestock, and timber cutting for 45 years, found TC, FC, and FS counts ranged to maxima of 570, 183, and 500 organisms per 100 ml, respectively. Kunkle (12) obtained similar results in runoff from a hayfield in a rural Vermont watershed that had not been grazed, manured, or fertilized for over 8 years; yet the TC and FC counts in runoff ranged to maxima of 16,000 and 1,000 organisms per 100 ml, respectively. Goodrich et al. (9) found that the elevated indicator counts in reservoir water of a closed watershed in Montana, as compared with those in a nearby reservoir with a contributing

TABLE 4. Influence of two incubation temperatures and time on the numbers of FC, FS, and percent starch-hydrolyzing FS, and FC/FS ratios of diluted cattle manure<sup>a</sup>

Temp/bacteriological indicator	Incubation time (h)						
	0	8	16	24	32	40	48
At 4°C							
FC <sup>b</sup>	4	7	5	7	4	8	9
FS <sup>b</sup>	4	4	4	4	3	5	10
Starch-hydrolyzing FS (%)	81	67	48	69	31	19	53
FC/FS ratio	1.1	1.9	1.7	1.9	1.2	1.7	0.8
At 21°C							
FC <sup>b</sup>	4	23	160	730	1,240	1,320	1,430
FS <sup>b</sup>	4	3	4	5	7	4	9
Starch-hydrolyzing FS (%)	81	63	17	24	3	1	4
FC/FS ratio	1.1	8.6	44.3	138.4	185.6	315.1	155.8

<sup>a</sup> Fresh manure samples were diluted  $10^{-2}$  to  $10^{-3}$  with sterilized runoff water to simulate bacterial concentrations commonly found in pasture runoff.

<sup>b</sup> FC and FS counts are expressed as millions of organisms per 100 ml.

watershed open to the public, resulted from the contributions of elk. Clearly, general bacterial indicator groups are not appropriate for distinguishing the separate impact of wildlife, domestic animals, or humans as nonpoint sources of fecal contamination.

Aside from distinguishing human from non-human pollution sources, the FC/FS ratio seems useful in determining the relative contributions of domestic cattle and wildlife in runoff from grassland area. In our study, FC/FS ratios in runoff from grazed pasture were 11 times higher than those in runoff from the ungrazed area. Meiman and Kunkle (15) found that the mean FC/FS ratio in stream water from a grazed watershed in North Central Colorado was six times higher than that in stream water of a paired, undisturbed watershed. These differences in FC/FS ratios are undoubtedly related to differences in FC and FS populations in the feces of different animal groups. Geldreich (7) summarized the information available on the FC/FS ratios of the feces of warmblooded animals, part of which follows: average for human feces, 4.3; cattle, sheep, and poultry, from 0.104 to 0.421; and wild animals (including rabbits, field mice, chipmunks, and birds), 0.0008 to 0.043. Apparently, FC/FS ratios for the feces of wild animals are at least 10-fold lower than those of domestic livestock.

The FC/FS ratio seems to be useful in evaluating the effects of cattle management and distribution on runoff water quality. FC/FS ratios between 0.7 and 4.0 may indicate situations where cattle are localized close to sampling or outflow points. The use of the FC/FS ratio to distinguish human from nonhuman pollution sources in stream and well waters has been questioned, because it requires a careful consideration of the age of wastes, and should not be used for wastes over 24-h old (8, 14). Our findings, however, would substantiate the conclusion by Feachem (6) that the increase in FC/FS ratio from an initially low value is related to differential die-off rates and is characteristic of domestic animal wastes.

We found the FC/FS ratio of runoff water containing fresh bovine feces increased from 1.1 to 315.1 within 40 h at 21°C (Table 4). This dramatic increase resulted predominantly from aftergrowth of FC (330-fold increase) since FS counts remained constant during the same time interval. These findings confirmed our original field observations that elevated FC/FS ratios could be expected in runoff containing fecal organisms of recent origin.

As suggested by Middaugh et al. (16), the presence of *S. bovis* in water is an indicator of

contributions from ruminant livestock since it is usually absent from or present in reduced numbers in the feces of wild animals (3, 7). Because *S. bovis* has a short half-life outside of its respective host (8, 14), its presence in water is also an index of how recently the waste was deposited. Our findings confirm this observation. Total FS counts changed very little in runoff containing fresh bovine feces. However, the numbers of starch-hydrolyzing FS (presumably *S. bovis*) decreased from 81% of the total FS population to less than 20% within 40 h at both 4 and 21°C.

In evaluating the available literature on FS populations in water impacted by cattle, we must pay careful attention to the media used for enumeration of FS and *S. bovis*. Many of the media commonly used for enumeration of FS are inappropriate for characterizing fresh cattle wastes because of reduced ability to recover *S. bovis*. Switzer and Evans (23) reported poor recovery of *S. bovis* from bovine fecal samples with M-enterococcus agar, SF broth, or KF broth. Middaugh (16) attributed such reduced recovery to the presence of levels of azide in these media which are inhibitory to *S. bovis*. PSE agar has a lower level of azide and has been shown to be most effective in recovery of *S. bovis* (13, 20, 23).

The choice of media is important for proper enumeration of FS in water where fecal materials from different sources are involved. For example, PSE agar would be an appropriate choice for counting FS in water where bovine livestock are the source. However, Brodsky and Schiemann (2) reported that KF agar was more effective than PSE agar in recovery of FS from sewage effluent on membrane filters. Investigators should carefully evaluate several media for enumeration of FS where inputs from sewage and domestic livestock or wildlife are expected. The improper selection of media may greatly alter the FC/FS ratio (11) and interpretation of the type of fecal pollution involved.

A major directive of the Federal Water Pollution Act of 1972 (P.L. 92-500), Sec. 208, to federal and state agencies is evaluation of the impact of land-use practices on water quality and implementation of abatement measures for nonpoint sources of pollution. Included in abatement measures is the use of "Best Management Practices." The worthy purpose of this legislation is that we become better stewards of our environment to preserve it for present and future generations; however, the indiscriminate implementation of this legislation could place unfair restrictions upon agricultural and other land-use practices. Our current recommended standards

for the bacteriological quality of water, using indicator groups, have served well in improving sanitary standards and in reducing pollution from point sources. However, our findings indicated that fecal contributions from wildlife alone can exceed recommended water quality standards. We should not expect runoff from livestock production land to meet water quality standards that cannot be met by land on which wildlife are the only source of fecal bacteria.

In many cases, good management of grazing livestock can reduce bacterial contamination of adjacent water bodies to levels which are typical of natural conditions (4, 22). An objective evaluation of different management practices, good or bad, will require the use of water quality indicators that will separate livestock inputs from those of wildlife.

The FC/FS ratio and numbers of *S. bovis* in pasture runoff are useful indicators for both identifying and evaluating the relative fecal contributions from domestic livestock and wildlife. These indicators are also valuable in determining the effectiveness of different livestock management practices for minimizing bacterial contamination of surface waters. Further research is needed to evaluate the application of these specific indicator groups for determining the specific contributions of wildlife and domestic livestock to nonpoint source pollution.

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