Numbers of fecal streptococci and *Escherichia coli* in fresh and dry cattle, horse, and sheep manure

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Abstract: Livestock are known contributors to stream pollution. Numbers of fecal streptococci and *Escherichia coli* in manure naturally deposited by livestock in the field are needed for activities related to bacterial source tracking and determining maximum daily bacterial loading of streams. We measured populations of fecal streptococci and *E. coli* in fresh and dry manure from cattle (*Bos taurus* L.), horses (*Equus caballus* L.), and sheep (*Ovis aires* L.) on farms in southern Idaho. Populations of indicator bacteria in dry manure were often as high as that in fresh manure from horse and sheep. There was a 2 $\log_{10}$ drop in the population of fecal coliform numbers in dry cattle manure from cattle in pastures but not from cattle in pens. Bacterial isolates used in source tracking should include isolates from both fresh and dry manure to better represent the bacterial source loading of streams.

Key words: enterococci, *E. coli*, fecal streptococci, bacterial indicators, bacterial source tracking, pollution.

Résumé : Il est reconnu que les animaux d'élevage contribuent à la pollution des cours d'eau. Un certain nombre d'entérococques, de streptocoques fécaux et *Escherichia coli* dans le fumier déposé naturellement par le bétail dans le champ sont nécessaires pour accomplir les activités reliées au repérage des sources et à l'estimation de la charge maximale bactérienne quotidienne dans les cours d'eau. Nous avons mesuré les populations de streptocoques fécaux et de *E. coli* dans le fumier frais et sec de vaches (*Bos taurus* L.), de chevaux (*Equus caballus* L.) et de moutons (*Ovis aires* L.) de fermes du sud de l'Idaho. Les populations de bactéries indicatrices dans le fumier sec étaient souvent aussi élevées que dans le fumier frais provenant de chevaux ou de moutons. Une chute de 2 $\log_{10}$ des populations de coliformes fécaux fut détectée dans le fumier sec de vaches provenant de pâturages mais non de vaches dans des enclos. Des isolats bactériens utilisés dans le repérage des sources devraient inclure des isolats aussi bien de fumier frais que sec afin de mieux représenter les sources de charge bactérienne dans les cours d'eau.

Mots clés : entérococques, *E. coli*, streptocoques fécaux, indicateurs bactériens, repérage de sources bactériennes, pollution.

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Introduction

Considerable interest has developed in determining the source of fecal contaminants reaching surface waters. A method used in the past was the ratio between fecal coliforms and fecal streptococcus (Geldreich and Kenner 1969; Geldreich 1976). The method has not proved to be reliable (APHA et al. 1998; Pourcher et al. 1991; Howell et al. 1996), and now other methods are sought based on phenotypic and genotypic characteristics of *Escherichia coli* or *Enterococcus* spp., a subgroup of fecal streptococcus (Scott et al. 2002). The methods generally require a library of isolates, obtained by isolating the target bacteria from samples of fecal material from known sources and comparing these with isolates from the water body of interest (Graves et al. 2002; Parveen et al. 2001; Solo-Gabriele et al. 2000). The different methodologies have shown a degree of success, but often many isolates from the water body cannot be definitively matched with the library of isolates from known sources (Hartel et al. 2002; Johnson et al. 2004; McLellan 2004; Wiggins et al. 2003).

The general method of obtaining isolates from known sources is to collect fecal samples directly from the animal or from fresh droppings (Graves et al. 2002; Barnes and Gordon 2004; Hartel et al. 2002; Johnson et al. 2004; Wheeler et al. 2002). The reason or reasons for not collecting from dry fecal material are not expressed, but one reason may be that populations of fecal indicators presumably would decline rapidly once leaving the animal and being deposited on the ground. Another reason may be that microbiologists are concerned about obtaining fecal samples that have not been contaminated by external sources, so they choose freshly deposited manure. If the recommended animal culture practices are followed to protect water resources, livestock would not be allowed direct access to surface water

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bodies, and thus the main reservoir of fecal material would be dry material.

The presence of livestock increases the numbers of indicator bacteria in runoff from watersheds (Doran and Linn 1979; Jawson et al. 1982; Khaleel et al. 1980; Stephenson and Street 1978; Tiedemann et al. 1988), and the numbers remain high long after the animals are removed (Jawson et al. 1982; Stephenson and Street 1978; Tiedemann et al. 1988). A controlled experiment using cattle manure demonstrated that there was only a tenfold decrease in populations of fecal coliforms released by 10 min of artificial rainfall onto hand-molded fecal deposits at 30 days in comparison to a fresh deposit (Thelin and Gifford 1983). The fecal deposits were hard after 2 days and completely dry by 15 days. The reduced release of the fecal coliforms with time may not have been due to die-off in the manure, which was not measured, but may have been due to the change in physical condition (Thelin and Gifford 1983) that did not allow the rainfall to readily disperse the manure.

Our interest was to measure the population size of *E. coli* and fecal streptococci in freshly deposited manure from cattle (*Bos taurus* L.), horse (*Equus caballus* L.), and sheep (*Ovis aires* L.) and these bacterial indicators in manure deposits dry to a dry state. In addition, with the recognition that diet influences the populations of fecal bacteria (Jarvis et al. 2000; Russell et al. 2000), manure from cattle in confined feeding and pastured were sampled. The study was conducted in southern Idaho, which has an arid climate with little precipitation to disturb fecal deposits during the summer.

Materials and methods

Study area

The study was conducted near Twin Falls, Idaho. The study area is located on the Snake River Plain, between 42°30′00′′ and 43°30′00′′N and between 114°20′00″ and 116°30′00′′W. The sites occur across an elevational gradient ranging from 860 to 1300 m. The area is classified as a temperate semi-desert ecosystem (Bailey 1998). The climate is typified by cool, moist winters and hot, dry summers with annual precipitation ranging from 175 to 305 mm, two-thirds of which occurs during October through March (Collett 1982). Average annual temperature ranges from 9 to 10 °C. During our investigation, daily maximum air temperature averaged 32.3±3.5 °C and daily minimum air temperature averaged 13.8±2.7 °C. Soils are typically well-drained loams and silt loams derived from loess deposits overlying basalt.

Pastures

Irrigated pastures were vegetated with various mixtures of Kentucky bluegrass (*Poa pratensis* L.), orchardgrass (*Dactylis glomerata* L.), and smooth brome (*Bromus inermis* Leyss.) orchardgrass. Furrow irrigation was practiced according to need for water.

Penned cattle

Dairy cattle were fed individual rations according to each cow to maximize milk production. They typically received a mixture of approximately 4.5 kg of alfalfa, 25 kg of corn silage, 5 kg of corn–oat mixture, 3.7 kg of a 44% sugar beet – 66% molasses mixture, 0.14 kg of decial containing 18% P, and 0.13 kg of salt and vitamins per day per cow.

Collection of manure

Manure samples were collected during July, August, and early September 2003 from the droppings of cattle, horses, and sheep for determining the population size of fecal streptococci and presumptive *E. coli* in manure. Manure was collected from cattle on two pastures, in a dairy, and in a feedlot. Three fresh manure patties and three dry patties were collected from each pasture. Three fresh manure patties and three dry patties were collected from the dairy and the feedlot.

Manure samples from horses were collected from manure in four pastures. Two fresh samples and two dry samples were collected from each pasture.

Manure samples from sheep were collected from droppings of sheep in three pastures or from nighttime penning areas adjacent to pastures. Two fresh samples and two dry samples were collected from each pasture.

Samples were collected using plastic disposable spoons. Approximately 100-g samples were placed in sterile plastic bags and taken to the laboratory for processing within 2 h of collection. On the day of collection, samples were not exposed to temperatures above 24 C.

Processing of manure samples

The fresh manure samples were thoroughly mixed in the plastic bags before 1 g was removed for determining the population size by membrane filtration. Approximately 20 g was removed for determining the water content by drying to constant weight at 102 °C. A fresh 1-g subsample for the population count was transferred to a 120-mL diluent bottle containing 99 mL of 0.31 mmol $KH_2PO_4·L^{-1}$ buffer and shaken vigorously by hand for approximately 30 s before making serial dilutions for plating by membrane filtration. Membranes were placed on m-Enterococcus agar and incubated for 48 h at 37 °C before counting red colonies typical of fecal streptococcus (Slanetz and Bartley 1957; APHA et al. 1998). A second membrane was placed on m-TEC agar (Difco, Detroit, Michigan) and incubated for 24 h at 44.5 °C in a water bath before counting yellow colonies typical of *E. coli* (APHA et al. 1998).

The dry samples were not easily mixed because they were dry. Subsamples were taken from different positions within the main sample for population counts and for determining moisture content. A mortar and pestle was used to macerate a 1-g subsample for determining population size. After grinding the 1-g subsample in 10 mL of 0.31 mmol $KH_2PO_4·L^{-1}$ buffer, the suspension was transferred to a 120-mL diluent bottle containing 90 mL of 0.31 mmol $KH_2PO_4·L^{-1}$ buffer and was shaken vigorously by hand for approximately 30 s before making serial dilutions for plating by membrane filtration, by the same procedure as used for the fresh samples.

Statistical evaluation

The population data from membrane filtration were adjusted according to the moisture content of samples to number per gram of dry mass for statistical analyses and reporting. For statistical analyses, the counts were transformed to $\log_{10}$. Analysis of variance was conducted on data using a factorial

Table 1. Number of fecal streptococci and *Escherichia coli* in fresh and dry manure from cattle on pasture or in pens.

Location	Manure	Fecal streptococci ($\log_{10}\cdot g^{-1}$)	*E. coli* ($\log_{10}\cdot g^{-1}$)	Significance
Pasture	Fresh	5.15	5.88	0.036 S
	Dry	4.68	3.81	0.042 S
Penned	Fresh	4.62	7.27	0.000 S
	Dry	5.78	6.06	0.937 NS

Note: S, significant difference at $p \leq 0.05$ between numbers of fecal streptococci and *E. coli*, based on a paired comparisons test. NS, not significant. The coefficient of variation from analysis of variance was 0.21.

design of treatments with indicator organism, pasture, or confined animals, and fresh or dry manure as factors in the case of cattle. For horses and sheep, there were only the two factors of indicator organism and fresh or dry manure.

In some cases, additional statistical analyses were conducted using a paired comparisons test to determine statistical significance of population differences of fecal streptococci and *E. coli* within fresh and dry manure samples from the same animal type. Because both populations were measured on the same manure sample, this method provided greater sensitivity for detecting significant differences between populations.

Results

Because both indicator organisms were measured from the same sample, it was possible to use pair-wise comparisons to determine significant differences between populations of fecal streptococci and *E. coli* within manure type and within location. For cattle, the pair-wise comparison showed that populations of *E. coli* were more numerous than populations of fecal streptococci in fresh manure (Table 1). The population of fecal streptococci was significantly higher than the population of *E. coli* in dry cattle manure in pasture but was not significantly different from dry cattle manure in pens.

Analysis of variance for populations of indicator bacteria in cattle manure indicated that the three-way interaction between locations (pasture or pen), manure type (fresh or dry), and indicator organism (fecal streptococci or *E. coli*) was not significant. An objective of our investigation was to determine the population difference between fresh and dry manure. The two-way interaction between manure type and indicator organism was statistically significant. Inspection of the data shows that the population size of *E. coli* was lower in dry manure than in fresh manure from pasture and pens, but the population of fecal streptococci was higher in dry manure from pens than in fresh manure from pens (Table 1).

Another interest was to determine if populations in manure of confined cattle operations were different form those of cattle on pasture. The populations were different, since analysis of variance indicated that there were statistically significant interactions between location (pens or pasture), indicator organism, and between location and manure type. Inspection of the data in Table 1 shows that the highest population in pens was over 1×10^6 *E. coli*·(g of fresh manure)$^{-1}$ in pens and was less than 1×10^6 *E. coli*·(g of fresh manure)$^{-1}$ from pasture. The range in population size for fecal streptococci in pasture and pens was not as large as for *E. coli*.

For horses, there was not a significant interaction between populations of the indicator organisms and manure type according to analysis of variance. The population size of fecal streptococci in horse manure was higher than the population size of *E. coli* (Table 2). The population size of the indicator organisms in fresh and dry manure was not significantly different according to analysis of variance.

The paired comparisons test for populations of indicator bacteria in manure from sheep indicated that the populations of fecal streptococci and *E. coli* were not significantly different (Table 3). Analysis of variance indicated that there was not a significant difference between populations of indicator bacteria in fresh and dry manure and that there was not a significant interaction between manure type and indicator bacterium.

Discussion

The populations of both indicator organisms in dry manure from all livestock was surprisingly high, since the manure would have been exposed to the environmental conditions long enough for it to become relatively dry. The moisture content of the fresh manure was 83%±3%, 78%±4%, and 74%±8% for cattle, horses, and sheep, respectively. The moisture content in dry manure was 12%±5%, 14%±11%, and 12%±6% for cattle, horses, and sheep, respectively. Samples collected as fresh were recently deposited because livestock were in near vicinity of the manure, and the manure had the consistency and appearance of being fresh. It would have been possible to select dry manure in various stages of drying, but only manure that appeared completely dry throughout the dropping was selected. Under the particular environmental conditions, it seemed that one to several weeks would be required to dry the manure to the point it was when sampled. In the neighboring state of Utah, Thelin and Gifford (1983) reported that after 15 days outside, an individual manure pile was dry throughout. Manure that was dry to the point of all green color being bleached out from weathering was not included in our study. Samples of such manure contained lower than 10 indicator bacteria·(g of manure)$^{-1}$.

Since the manure was collected after falling to the ground, there may be some concern of contamination from the underlying soil. In other studies using the same soil types and bacteriological media, Entry et al. (2005) reported zero col-

Table 2. Number of fecal streptococci and *Escherichia coli* found in fresh and dry manure from horses on pasture.

Indicator	Fresh ($log_{10}·g^{-1}$)	Dry ($log_{10}·g^{-1}$)
Fecal streptococci	5.47	6.14
E. coli	4.79	5.08
Significance	0.033 S	0.035 S

Note: S, significant difference at $p \leq 0.05$ between numbers of fecal streptococci and *E. coli* based on a paired comparisons test. The coefficient of variation based on analysis of variance was 0.17.

Table 3. Number of fecal streptococci and *Escherichia coli* found in fresh and dry manure from sheep on pasture.

Indicator	Fresh ($log_{10}·g^{-1}$)	Dry ($log_{10}·g^{-1}$)
Enterococcus spp.	4.96	6.25
E. coli	6.05	5.63
Significance	0.368 NS	0.384 NS

Note: NS, no significant difference at $p \leq 0.05$ between numbers of fecal streptococci and *E. coli* based on a paired comparison test. The coefficient of variation from analysis of variance was 0.22.

ony forming units of *E. coli* from soil not receiving animal manure. They also reported that the soil contained between 0 and 350 fecal streptococci·(g of soil)$^{-1}$. These numbers were so much smaller than the numbers we found in manure that any contamination from soil would not have been significant.

The population size of *E. coli* in the fresh manure from cattle on pasture or in pens was significantly different in our study (Table 1). In contrast, Aslam et al. (2003) did not find a significant difference among 10 cattle in pasture and the same 10 cattle in a feedlot. The populations ranged between approximately 5.5 and 6.0 log_{10}·(g of manure)$^{-1}$ for all sampling times across both environments. The reason for the different results is not clear but may be due to animal ages and types. The cattle in their study were primarily beef breeds of a uniform age. In our study, most of the cattle were Holsteins used for milk production and varied in age from 6- to 8-month-old calves to mature cattle in pasture to primarily older cattle in pens. There was an expectation by us that a change in diet from pasture grass to feed grains, alfalfa hay, and corn silage would make a difference in population size. It is known that a change in diet of cattle may alter the composition of the intestinal microbial flora (Jarvis et al. 2000; Russell et al. 2000). Jarvis et al. (2000) reported that a change in diet from predominantly grass hay to grain increased the population of *E. coli* in the colon of Holstein cattle from 4.3 to 7.7 log_{10}·(g of colon contents)$^{-1}$. The population of *E. coli* in fresh manure from 12-month-old Brahman cattle in a feedlot in Australia averaged 5.8 $log_{10}·g^{-1}$ but ranged between 3 and 7.56·g^{-1} (Midgley and Desmarchelier 2001).

The large decrease in numbers of *E. coli* in the dry manure samples from pastured cattle in comparison to penned cattle was not expected (Table 1). It has been hypothesized that the physical changes of crusting in a manure deposit from cattle would help protect the bacteria from the environment (Thelin and Gifford 1983). In our study, the dry manure deposits in the field were intact, but in the pens they were often ground under the hooves of the cattle and were thus not intact. It may be that in the pens the dry manure was continually being mixed with fresh manure from the hooves of the cattle and thus re-inoculated. The moisture content of the dry manure from pens and pasture was not significantly different. The numbers of fecal streptococci did not significantly decrease in the dry manure in comparison to fresh manure, which indicates the relatively poor ability of *E. coli* to survive outside the animal under conditions of variable temperature and moisture (Reddy et al. 1981; Winfield and Groisman 2003). The numbers of *E. coli* were higher than the numbers of fecal streptococci in fresh samples, which confirms the results of Pourcher et al. (1991), in which they point out the fallacy of expecting the ratio of fecal coliforms to fecal streptococcus to be less than one.

Both indicator organisms survived well in dry manure from horse (Table 2) and sheep (Table 3). The populations of fecal streptococci and *E. coli* in fresh manure from horse and sheep were comparable to the numbers reported by Pourcher et al. (1991). The lack of statistical significance between a one log higher population of fecal streptococci in dry sheep manure than in fresh manure is an indication of the considerable variability between manure deposits as has been reported by Pourcher et al. (1991) for fresh manure samples.

Pollution of surface flow and ground water from animal waste applied to soils has been documented (Mallin et al. 1997; Mawdsley et al. 1995). Solid livestock deposited on land can become liquid waste after rainfall or irrigation, and solute and microbe movement into the soil will follow ground water drainage patterns, which can potentially contaminate adjoining surface water. These same bodies of water are often sources of drinking water or are used for recreational activities. Human contact with recreational waters containing intestinal pathogens is an effective method of disease transmission. We found that *E. coli* and fecal streptococci survive well in dry manure samples from cattle, horse, and sheep. Our results may help explain why streams may continue to be polluted by fecal bacteria long after the livestock have been removed. It also raises concerns about only sampling fresh manure for isolation of bacteria used in bacterial source tracking, since the reservoir of indicator bacteria would be much greater in dry manure since it is more plentiful. There has already been considerable interest in the diversity of these bacteria isolated from fresh manure of various animal types and the impact of time, diet, environment, and geographic location on diversity (Aslam et al. 2003; Barnes and Gordon 2004; Hartel et al. 2002; Johnson et al. 2004). It is likely that including bacterial isolates from dry manure may increase the number of isolates from streams definitively matching with bacterial isolates from animals in bacterial source tracking.

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