

## Prevalence and Impact of Bacteriophages on the Presence of *Escherichia coli* O157:H7 in Feedlot Cattle and Their Environment<sup>∇</sup>

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The relationship between endemic bacteriophages infecting *E. coli* O157:H7 (referred to as “phage”) and levels of shedding of *E. coli* O157:H7 by cattle was investigated in two commercial feedlots in southern Alberta, Canada. Between May and November 2007, 10 pens of cattle were monitored by collection of pooled fecal pats, water with sediment from troughs, manure slurry from the pen floor, and rectal fecal samples from individual animals (20 per pen) at two separate times. Bacteriophages infecting *E. coli* O157:H7 were detected more frequently ( $P < 0.001$ ) after 18 to 20 h enrichment than by initial screening and were recovered in 239 of 855 samples (26.5% of 411 pooled fecal pats, 23.8% of 320 fecal grab samples, 21.8% of 87 water trough samples, and 94.6% of 37 pen floor slurry samples). Overall, prevalence of phage was highest ( $P < 0.001$ ) in slurry. Recovery of phage from pooled fecal pats was highest ( $P < 0.05$ ) in May. Overall recovery did not differ ( $P > 0.10$ ) between fecal grab samples and pooled fecal pats. A higher prevalence of phage in fecal pats or water trough samples was associated ( $P < 0.01$ ) with reduced prevalence of *E. coli* O157:H7 in rectal fecal samples. There was a weak but significant negative correlation between isolation of phage and *E. coli* O157:H7 in fecal grab samples ( $r = -0.11$ ;  $P < 0.05$ ). These data demonstrate that the prevalence of phage fluctuates in a manner similar to that described for *E. coli* O157:H7. Phage were more prevalent in manure slurry than other environmental sources. The likelihood of fecal shedding of *E. coli* O157:H7 was reduced if cattle in the pen harbored phage.

Bacteriophages are the most abundant biological entities on earth. An estimated  $10^{30}$  marine bacteriophages are harbored in the ocean, and they significantly influence microbial communities and function (27). As resistance is an increasing challenge in antimicrobial therapy, the antimicrobial nature of bacteriophages is being more intensively studied (13, 15). Bacteriophages naturally inhabit the mammalian gastrointestinal tract (1, 8), and *Escherichia coli*-infecting bacteriophages are commonly isolated from sewage, hospital wastewater, and fecal samples from humans and animals (3). Ruminants have been shown to shed up to  $10^7$  bacteriophage per gram of feces (6), and in humans multiple types of bacteriophage exhibiting activity against *E. coli* have been isolated from a single fecal sample (7).

*E. coli* O157:H7 is an important zoonotic bacterium carried asymptotically by cattle and readily isolated from manure, manure slurry, and drinking water in dairies and feedlots (11, 24, 30). Additionally, *E. coli* O157:H7 shedding by cattle has a seasonal pattern, peaking in the summer months (2, 25). Bacteriophage strains that infect *E. coli* O157:H7 have also been isolated from animal feces and have shown lytic activity against this bacterium in vivo and in vitro (5, 23, 28, 31). In recent studies, such phages were shown to be widely distributed in

cattle and in feces on the pen floor within feedlots (4, 18). However, the relationships between the presence of *E. coli* O157:H7-infecting bacteriophage in cattle and their environment and the shedding of this bacterium by cattle are largely undefined. Consequently, the aims of the present study were (i) to determine the prevalence of endemic *E. coli* O157:H7-infecting bacteriophage (referred to as “phage”) in feedlots over a 7-month period and (ii) to compare the presence of phage to the occurrence of *E. coli* O157:H7 in cattle and their environment.

### MATERIALS AND METHODS

**Study design and feedlot description.** The study was conducted using 10 pens of cattle (18 to 24 months old; average initial weight, 524 kg) in two commercial feedlots (pens 1 to 4 in feedlot A and pens 5 to 10 in feedlot B) from May to November 2007. The average stocking density during this study was 174 head per pen. Pooled fecal pats were collected from 10 pens, as shown in Fig. 1. Samples were collected only when animals were present in the pens and at times when feedlot operators would grant access to the premises.

**Sample collection.** To evaluate phage presence and *E. coli* O157:H7 shedding by individuals, rectal fecal samples were collected on two occasions from 20 cattle in each of pens 1 to 8. Approximately 10 g of feces per animal was collected by digital retrieval into a sterile Whirl-Pac bag (VWR International, Edmonton, Alberta, Canada). Samples were collected midway through the feeding period (S1) and just prior to shipment to market (S2) (Fig. 1). For S1 samples, each pen was sampled on a single visit, between May and August. Regarding the S2 samples, cattle in feedlot A were marketed in different groups, and thus, several sampling dates were required to collect individual fecal samples from all 20 animals within each pen, whereas at feedlot B, all cattle within a pen were marketed on the same date, and thus, samples from all 20 animals could be collected in a single visit to the feedlot.

Environmental samples from these eight pens were collected once weekly for at least two weeks before collection of fecal grab samples (Fig. 1). Environmental

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TABLE 1. Likelihood of recovery of phage-positive samples among those collected from two feedlots between May and November 2007<sup>a</sup>

Sample type <sup>b</sup>	No. of positive samples/no. tested (%) <sup>c</sup>			Odds ratio	95% confidence interval	P value
	Feedlot A	Feedlot B	Total			
PAT	69/214 (32.2)	40/197 (20.3)	109/411 (26.5)	0.02	0.02–0.06	<0.001
FEC	36/160 (22.5)	40/160 (25.0)	76/320 (23.8)	0.02	0.01–0.05	<0.001
WAT	8/46 (17.4)	11/41 (26.8)	19/87 (21.8)	0.02	0.01–0.05	<0.001
SLU	21/23 (91.3)	14/14 (100)	35/37 (94.6)			

<sup>a</sup> The percentage of phage-positive samples in slurry samples was used as the referent.

<sup>b</sup> PAT, pooled fecal pats; FEC, rectally collected fecal material; WAT, water with sediment from troughs; SLU, manure slurry.

<sup>c</sup> The status of samples was determined by initial screening and/or enrichment followed by plaque assay.

firmed isolates. The Cohen kappa statistic ( $\kappa$ ) was used to assess agreement between initial screening and enrichment methods. Correlations between isolation of *E. coli* O157:H7 and phage were assessed using the Pearson product-moment correlation coefficient. Odds ratios were generated for detection of phage using the GLIMMIX procedure. All analyses were conducted using the SAS system for Windows, version 9.1 (21).

**RESULTS**

**Prevalence of phage.** Bacteriophages active against *E. coli* O157:H7 were found in all types of samples collected from all pens at feedlots A and B (Table 1). Overall, 239 (28.0%) of all 855 samples were positive for phage by initial screening and/or enrichment. The prevalence of phage in pats was higher ( $P < 0.05$ ) in feedlot A (32.2%) than in feedlot B (20.3%), whereas no difference in rate of detection of phage in rectal fecal samples was observed between feedlots A (22.5%) and B (25.0%).

Bacteriophages active against *E. coli* O157:H7 were isolated from 19 (21.8%) of 87 trough samples, with no difference between feedlots ( $P = 0.56$ ). Eight of the 10 pens yielded phage-positive trough samples at least once. In general, phage were isolated more frequently from troughs in May than in other months (Table 2). The percentage of slurry samples positive for phage (94.6%) was greater than was observed for other sample types ( $P < 0.001$ ), with no difference between feedlots ( $P = 1.0$ ). All slurry samples from feedlot B were phage positive. Slurry samples were mainly available in May and sporadically in July after a period of wet weather (Table 2). Consequently, collection of slurry was possible only in 9 of 10 pens during the period from May to October. Odds ratios comparing the frequencies of detection of phage in fecal pats, rectal fecal material, or trough sample with frequency of de-

tecting phage in slurry (Table 1) indicated that the former three sample types were 98% less likely ( $P < 0.001$ ) than slurry to be phage positive.

**Variation of phage prevalence in pooled fecal pats over time.** At both feedlots, the prevalence of phage in fecal pat samples was greatest ( $P < 0.05$ ) in May (Fig. 2). At feedlot A, phage were more frequently detected in fecal pats in May (78.6%) than in August, September, or October ( $P < 0.01$ ), and prevalence was higher ( $P < 0.05$ ) in July (50.0%) and September (32.3%) than in August. At feedlot B, phage were more frequently ( $P < 0.05$ ) detected in pats in May (59.1%) than in July (10.9%), September (8.9%), or October (5.0%).

The prevalence of phage in fecal pats in May (67.7%) was higher ( $P < 0.001$ ) than that in any other month. Prevalence declined to 24.4% in July and 26.0% in August. In September, phage were isolated from only 16.6% of fecal pats, but this value was not different ( $P = 0.59$ ) from prevalences in July and August.

**Prevalence of *E. coli* O157:H7 and phage.** Across samplings S1 and S2, the prevalence of phage in rectal fecal samples (20 per pen) ranged from 5.0% to 47.5% (Table 3). Interestingly, 19 of 20 animals from pen 2 (feedlot A) were positive for phage at S1 in June, but all 20 animals from this pen were negative for phage at S2 in September and October.

Pooled fecal pats collected within 16 days of rectal fecal grab sampling were used to compare the frequencies of phage in pooled fecal pats and rectal fecal samples during S1 and S2 (Table 3). Overall, the prevalences of phage in these two sample types did not differ across sampling times, except for pen 4 in feedlot A, where the prevalence of phage was higher ( $P < 0.01$ ) in pats from the pen floor than in rectal fecal material.

Pooled fecal pats and trough samples collected within 16 days of fecal grab sampling were used to determine the impact of phage on the prevalence of *E. coli* O157:H7 in cattle (rectal fecal samples at S1 and S2) (Fig. 1; Table 3). Across the two time periods and feedlots, the prevalence of *E. coli* O157:H7 in rectal fecal samples was 38.8% (124 of 320). Overall, phage were detected in 56 (26.4%) of 212 fecal pats and 10 (22.7%) of 44 trough samples collected just prior to S1 and S2. Among the eight pens monitored, higher prevalence of phage in pats or trough samples was associated ( $P < 0.01$ ) with reduced prevalence of *E. coli* O157:H7 in fecal grab samples.

Analysis of the prevalence of *E. coli* O157:H7 and phage in rectal fecal samples revealed that 16.9% of 320 fecal samples were culture negative for *E. coli* O157:H7 but positive for phage, and only 6.9% were positive for both *E. coli* O157:H7 and phage. Isolation of phage was 43% less likely

TABLE 2. Monthly prevalence of phage-positive environmental samples collected from feedlots between May and November 2007<sup>a</sup>

Month	No. of positive samples/no. tested		
	PAT	WAT	SLU
May	24/36	9/12	14/14
June	4/20	0/4	1/1
July	26/95	6/19	9/9
August	47/70	0/14	6/8
September	25/110	2/22	4/4
October	13/75	1/15	1/1
November	3/5	0/1	NS

<sup>a</sup> Phage refers to naturally occurring bacteriophage capable of infecting *E. coli* O157:H7. Sample status was determined by initial screening and/or by enrichment followed by plaque assay. PAT, pooled fecal pats; WAT, water with sediment from troughs; SLU, pen floor manure slurry; NS, no sample available.

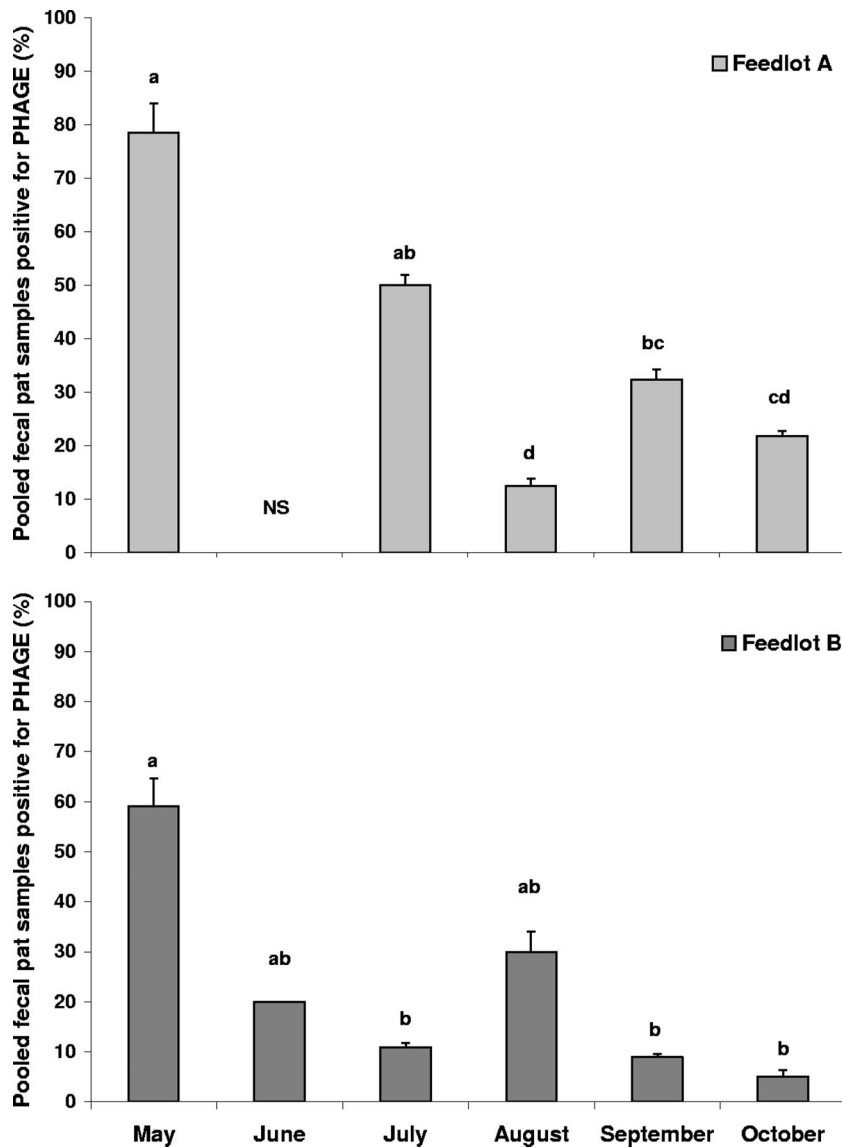


FIG. 2. Percentage, across pens, of pooled fecal pats testing positive for bacteriophage infecting *E. coli* O157:H7 between May and October in feedlots A and B, by the initial screening method and/or by enrichment followed by plaque assay. Error bars indicate standard errors. Within a feedlot, means with different letters (a to d) differ significantly ( $P < 0.05$ ). No samples were collected in June at feedlot A (NS).

(95% confidence interval = 0.32 to 0.99;  $P < 0.05$ ) from *E. coli* O157:H7 culture-positive fecal samples than from those testing negative. Bacteriophage were isolated more ( $P < 0.05$ ) frequently from samples testing positive for *E. coli* O157:H7 via direct plating (12 of 42) than from those testing positive via IMS (10 of 82). Populations of *E. coli* O157:H7 in phage-positive rectal fecal samples averaged 4.6 log<sub>10</sub> CFU/g by direct plating. In contrast to the results for rectal fecal samples, isolation of phage across sampling times and feedlots was unrelated ( $P > 0.1$ ) to detection of *E. coli* O157:H7 in fecal pats or water trough samples.

**Initial screening versus enrichment for phage detection.** Agreement between initial screening and enrichment for detecting phage was fair ( $\kappa = 0.29$  to 0.39) within and across feedlots, irrespective of sampling type or time, and was

slight to fair among different sample types ( $\kappa = 0.19$  to 0.26) across feedlots. Overall, the proportions of samples testing phage positive without enrichment were 27 of 411 fecal pats (6.6%), 18 of 320 fecal grab samples (5.6%), 10 of 87 water trough samples (11.5%), and 28 of 37 slurries (75.7%). The percentage of slurry samples testing phage positive by initial screening was higher ( $P < 0.001$ ) than that of other sample types, which did not differ from one another. Enrichment increased detection rates by 20.0%, 18.1%, 10.3%, and 18.9% for pats, rectal feces, trough samples, and slurry samples, respectively. In total, enrichment detected approximately twice as many phage-positive isolates as did initial screening (156 versus 83).

Odds ratios comparing detection of phage by initial screening and enrichment are presented in Table 4. Across

TABLE 3. Prevalence of *E. coli* O157:H7 and of bacteriophage infecting *E. coli* O157:H7 (phage) in samples<sup>a</sup> collected from two southern Alberta feedlots

Organism type and sample origin	No. of animals per pen	Prevalence of organism (no. of samples positive <sup>b</sup> /no. of samples tested)								
		S1 <sup>c</sup>			S2 <sup>c</sup>			Total		
		PAT	WAT	FEC	PAT	WAT	FEC	PAT	WAT	FEC
<i>E. coli</i> O157:H7 <sup>d</sup>										
Feedlot A										
Pen 1	152	0/2	0/2	9/20	0/2	0/3	12/20	0/4	0/5	21/40
Pen 2	152	0/2	0/2	0/20	2/4	0/4	11/20	4/6	0/6	11/40
Pen 3	253	0/2	2/2	12/20	1/6	0/6	14/20	1/8	2/8	26/40
Pen 4	153	1/2	1/2	10/20	1/7	0/7	4/20	2/9	1/9	14/40
Feedlot B										
Pen 5	235	1/1	2/2	12/20	0/2	0/2	13/20	1/3	2/4	25/40
Pen 6	151	0/2	2/2	12/20	2/2	0/2	12/20	2/4	2/4	24/40
Pen 7	138	2/2	1/2	15/20	1/2	0/2	2/20	3/4	1/4	17/40
Pen 8	149	0/2	1/2	15/20	0/2	0/1	4/20	0/4	1/3	19/40
Phage										
Feedlot A										
Pen 1	152	1/2	1/2	7/20	5/15	0/3	0/20	6/17	1/5	7/40
Pen 2	152	9/10	2/2	19/20	1/20	1/4	0/20	10/30	3/6	19/40
Pen 3	253	2/10	0/2	3/20	12/30	0/6	5/20	14/40	0/8	8/40
Pen 4	153	7/10	2/2	1/20	9/35	0/7	1/20	16/45	2/9	2/40
Feedlot B										
Pen 5	235	0/10	0/2	3/20	0/10	0/2	3/20	0/20	0/4	6/40
Pen 6	151	4/10	0/2	6/20	0/10	1/2	13/20	4/20	1/4	19/40
Pen 7	138	0/10	0/2	7/20	3/10	0/2	4/20	3/20	0/4	11/40
Pen 8	149	2/10	2/2	1/20	1/10	0/2	3/20	3/20	3/4	4/40

<sup>a</sup> PAT, pooled fecal pats; WAT, water with sediment from troughs; FEC, rectally collected fecal material. In each pen, the PAT and WAT samples were collected during the 16 days immediately preceding each of the two FEC samplings.

<sup>b</sup> The presence of *E. coli* O157:H7 was determined by enrichment and IMS. The presence of phage was determined by initial screening and/or by enrichment followed by plaque assay.

<sup>c</sup> Rectal fecal samples were collected on two occasions (in eight pens; 20 animals per pen) to evaluate *E. coli* O157:H7 and phage shedding by individuals. FEC samples were collected during cattle processing during implant of growth promotant (S1) and at final weigh-out prior to slaughter (S2), an interval of approximately 3 to 4 months.

<sup>d</sup> Assessment for *E. coli* O157:H7 in PAT samples was conducted on one randomly selected PAT from the five prepared per pen.

sample types, overall isolation of phage was 69.0% less likely ( $P < 0.001$ ) by initial screening than after enrichment. As well, across feedlots and sampling times, initial screening was 79.0 to 80.0% ( $P < 0.001$ ) less likely than enrichment to recover phage either from pooled fecal pats or from rectal fecal samples.

**DISCUSSION**

This study was the first to monitor over time the prevalence of phage in cattle and their environment. Bacteriophages targeting *E. coli* O157:H7 were detected in 26.5% of fecal pats and 23.8% of rectal fecal samples. These results confirm earlier

TABLE 4. Likelihood of detecting *E. coli* O157:H7-infecting bacteriophage from feedlot samples using initial screening compared to using enrichment

Sample type <sup>a</sup>	No. of samples				Odds ratio	95% confidence interval	P value
	Tested	Positive					
		Total	By initial screening				
PAT	411	109	27	102	0.21	0.14–0.33	<0.001
FEC	320	76	18	73	0.20	0.12–0.35	<0.001
WAT	87	19	10	13	0.74	0.30–1.80	0.50
SLU	37	35	28	33	0.38	0.10–1.39	0.14
Feedlot A	443	134	39	127	0.24	0.16–0.35	<0.001
Feedlot B	412	105	44	94	0.40	0.27–0.60	<0.001
Total (A+B)	855	239	83	221	0.31	0.23–0.41	<0.01

<sup>a</sup> PAT, pooled fecal pats; FEC, rectal fecal samples; WAT, water with sediment from trough; SLU, manure slurry.

findings (4, 18) that phage active against *E. coli* are likely ubiquitous within feedlots. Using the standard lab strain *E. coli* B as a host, Callaway et al. (4) isolated phage from 15% of rectal fecal samples and from 55% of feedlot pens. Oot et al. (18) reported that 65% of fecal pats in 10 pens were phage positive after enrichment using *E. coli* O157:H7 NCTC 12900 and *E. coli* B as hosts. In contrast to these two point-prevalence studies, we conducted a longitudinal investigation over months when shedding of *E. coli* O157:H7 was expected to be highest. This study is also the first to document isolation of phage from fecal slurry (94.6%) and water troughs (21.8%), demonstrating the wide distribution of phage in the feedlot environment.

In the present study, we selected *E. coli* O157:H7 R508 as the host strain for phage detection because it is a member of phage type 14. In Canada, phage types 14 and 14a are the predominant phenotypes of bovine and nonbovine *E. coli* O157:H7 isolates, and both have been associated with numerous disease outbreaks (36; R. Ahmed and W. Demczuk, personal communication). It was hypothesized that this approach would increase the likelihood of our isolating phage that exhibited activity against those strains of *E. coli* O157:H7 that are most likely to cause human clinical disease. However, this selective approach may also have restricted our ability to isolate active phage against other strains or phage types of *E. coli* O157:H7. We are in the process of screening the 255 phage isolates obtained in the present study against our collection of ruminant and human *E. coli* O157:H7 isolates. Phage isolates that exhibit a low multiplicity of infection in a wide range of *E. coli* O157:H7 hosts will be selected for further study.

The frequency of phage isolation was noticeably high among slurry samples, with this detection frequently occurring without enrichment. Slurry comprises a mixture of urine, feces, water, spilled feed, and bedding (37). Slurry is high in moisture and nutrient content (9, 22), conditions that could promote host growth and the proliferation and persistence of phage (34). Slurry could serve as a phage reservoir and may contribute to a reduction of the prevalence of *E. coli* O157:H7 within a pen, although the benefits of the presence of a phage-rich slurry would have to be balanced against the reduced growth of cattle housed in wet pens (26).

The present data indicate that the prevalence of phage in fecal pats was greatest in May. April and May were the two wettest months of 2007; June, July, and August were drier ([http://www.climate.weatheroffice.ec.gc.ca/advanceSearch/searchHistoricData\\_e.html](http://www.climate.weatheroffice.ec.gc.ca/advanceSearch/searchHistoricData_e.html)). Previous studies suggested that soilborne bacteriophages may persist longer in wetter environments (12, 33). Presumably, phage persistence in May could have been enhanced in the well-moistened fecal pats or the fecal slurry that was present in the feedlot pens. More frequent isolation of phage from water troughs in May also suggests a higher overall environmental prevalence of phage during this month. The prevalence of phage in feedlot A also increased from 12.5% in August to 32.3% in September, a period that coincided with an increase in rainfall (12.3 to 46.3 mm).

Interestingly, July at feedlot A showed the second highest prevalence of phage among the months sampled. July had the highest average ambient temperature (22.8°C), a factor that could also lead to increased growth of *E. coli* O157:H7 within the feedlot environment (2, 29) and, possibly, to the proliferation of phage. Higher isolation rates during warmer months

have also been reported for bacteriophages against O149 enterotoxigenic *E. coli* (14). The monthly variation of phage in fecal pat samples was consistent with the predator-prey relationship that likely exists between phage and their host both in cattle and their environment. When *E. coli* O157:H7 was abundant, phage increased and *E. coli* O157:H7 populations subsequently declined. With low numbers of host, phage declined, leading to a recovery of the *E. coli* O157:H7 population. The role that these fluctuations in predator-prey relationships play in the seasonal prevalence of *E. coli* O157:H7 (2, 25) remains to be determined.

In contrast to feedlot A, phage prevalence in feedlot B did not demonstrate the same monthly fluctuation, possibly because of more frequent turnover of animals within pens. Moreover, the pens in feedlot B were apparently better drained, given their less frequent accumulation of slurry compared with feedlot A. These conditions may have resulted in a lower overall prevalence of phage in feedlot B than in feedlot A. In seven of the eight pens, the overall prevalence of phage was similar between fecal pats and rectal fecal samples across two sample times (S1 and S2), suggesting that the shedding of phage from individual animals contributed directly to the observed prevalence in pats on the pen floor. Thus, for the majority of pens, there was no difference in the prevalence of phage in fecal pats versus rectal fecal grab samples.

The association between phage prevalence in fecal pats or water trough samples and reduced *E. coli* O157:H7 prevalence in rectal fecal material indicates that phage in the environment may reduce *E. coli* O157:H7 populations. Williams Smith et al. (35) demonstrated that treatment of litter (10<sup>2</sup> organisms/12 m<sup>2</sup> floor area) with phage active against enteropathogenic *E. coli* prevented diarrhea in calves. Sheng et al. (23) added phage to drinking water at 10<sup>6</sup> PFU/ml and suggested that this practice reduced the shedding of *E. coli* O157:H7 in cattle. Thus, administering phage on pen floors or in water troughs may be a convenient and effective means of controlling *E. coli* O157:H7 populations in cattle. The negative correlation between phage isolation and populations of its host *E. coli* O157:H7 in rectal fecal samples is in accord with the work of Oot et al. (18) and suggests that endemic phage attenuate populations of *E. coli* O157:H7. This study used a random subset of rectal fecal samples collected from a much larger investigation of *E. coli* O157:H7 in feedlots A and B (T. P. Stephens, unpublished data). In this study, the overall prevalence of *E. coli* O157:H7 in fecal grab samples (23.9%) was high but not unlike that reported previously (20). The high numbers of host persisting during the study period likely increased phage prevalence. Callaway et al. (5) demonstrated that naturally occurring phage isolated from cattle were capable of reducing *E. coli* O157:H7 populations in experimentally inoculated sheep. In the present study, phage isolation from rectal fecal material was more frequent in association with greater (4.6 log<sub>10</sub> CFU/g) than with smaller (<1.0 log<sub>10</sub> CFU/g) populations of *E. coli* O157:H7, which supports the theory of greater amplification of phage when more host is present. An in vitro study with bacteriophage T4 that exhibited activity against *E. coli* showed that amplification did not occur when numbers of the host dropped below 10<sup>3</sup> cells/ml (32). Consequently, *E. coli* O157:H7 may persist within cattle or the environment when phage proliferation is limited due to inadequate hosts, inap-

appropriate physiological states for replication, or an infrequent contact with target (7). Failure of phage to eliminate *E. coli* O157:H7 in cattle (17, 23) may be a result of suboptimal host-to-phage ratios. Callaway et al. (5) found that an initial ratio (1:1) of phage to host was most effective for reducing numbers of *E. coli* O157:H7 in cecal and rectal contents from sheep, illustrating that phage efficacy depends on the density of host cells.

Dilution of *E. coli* O157:H7 and phage may reduce the effectiveness of this approach for controlling this pathogen in large volumes of water. Our laboratory showed previously that bacteriophage are not effective in controlling *E. coli* O157:H7 inoculated into water microcosms (16). Even though phage was not effective in controlling *E. coli* O157:H7 in water, Sheng et al. (23) found that phage administered through water decreased shedding of *E. coli* O157:H7 by cattle. The necessity of host-phage interaction may make phage therapy more effective at mitigating *E. coli* O157:H7 in the animal than in the environment.

Improved detection of phage after enrichment compared to initial screening confirmed the findings of Oot et al. (18) and also showed that low levels of phage may persist within the environment. Given its utility for detecting phage-positive samples, the enrichment technique is strongly recommended as a means of determining prevalence of endemic phage in cattle and their environment.

In conclusion, this study demonstrated monthly fluctuations in populations of *E. coli* O157:H7-infecting bacteriophage. Bacteriophage against *E. coli* O157:H7 were more commonly isolated from pen floor slurry than from other sources examined. Bacteriophages isolated from the feedlot environment may prove to be efficacious for controlling *E. coli* O157:H7 in feedlot cattle. Observations in this study suggest that environmental factors such as moisture levels and temperature likely influence the prevalence of *E. coli* O157:H7-infecting phage. The prevalence of these bacteriophages in fecal pats from the pen floor was similar to that in fecal samples collected from the rectum. Most importantly, endemic phage both in individual animals and in their environment were associated with a reduced prevalence of *E. coli* O157:H7 in cattle. Increased knowledge of the ecology of phage could aid in refining this therapy as a means of controlling *E. coli* O157:H7 in feedlot cattle. Further work is required to define the genetic diversity of *E. coli* O157:H7-infecting bacteriophage isolates within pens and over time, as well as the degree of infectivity of these phages for a variety of strains of the host *E. coli* O157:H7.

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