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Differing Populations of Endemic Bacteriophages in Cattle Shedding High and Low Numbers of *Escherichia coli* O157:H7 Bacteria in Feces

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The objectives of this study were to identify endemic bacteriophages (phages) in the feedlot environment and determine relationships of these phages to *Escherichia coli* O157:H7 from cattle shedding high and low numbers of naturally occurring *E. coli* O157:H7. Angus crossbred steers were purchased from a southern Alberta (Canada) feedlot where cattle excreting $\geq 10^4$ CFU · g^{-1} of *E. coli* O157:H7 in feces at a single time point were identified as supershedders (SS; *n* = 6), and cattle excreting $< 10^4$ CFU · g^{-1} of feces were identified as low shedders (LS; *n* = 5). Fecal pats or fecal grabs were collected daily from individual cattle for 5 weeks. *E. coli* O157:H7 in feces was detected by immunomagnetic separation and enumerated by direct plating, and phages were isolated using short- and overnight-enrichment methods. The total prevalence of *E. coli* O157:H7 isolated from feces was 14.4% and did not differ between LS and SS ($P = 0.972$). The total prevalence of phages was higher in the LS group (20.9%) than in the SS group (8.3%; $P = 0.01$). Based on genome size estimated by pulsed-field gel electrophoresis and morphology determined by transmission electron microscopy, T4- and O1-like phages of *Myoviridae* and T1-like phage of *Siphoviridae* were isolated. Compared to T1- and O1-like phages, T4-like phages exhibited a broad host range and strong lytic capability when targeting *E. coli* O157:H7. Moreover, the T4-like phages were more frequently isolated from feces of LS than SS, suggesting that endemic phages may impact the shedding dynamics of *E. coli* O157:H7 in cattle.

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Escherichia coli (*E. coli*) O157:H7 is a foodborne organism responsible for severe illness and death in humans (1). Cattle are asymptomatic carriers of *E. coli* O157:H7, and contamination of foods through production and distribution chains has resulted in massive recalls and substantial economic loss to the food industry (2, 3). Shedding of *E. coli* O157:H7 is inconsistent, with some cattle shedding the organism for a few days while others may shed *E. coli* O157:H7 for weeks or months (4), suggesting that the nature of the host plays a role in the etiology of this bacterium. Supershedders are cattle that excrete the pathogen at concentrations of $\geq 10^4$ CFU · g^{-1} of feces (5); they are thought to account for a small proportion of the cattle population and yet may contribute 80 to 96% of the total load of *E. coli* O157:H7 in the herd (5–7). Multiple factors contribute to shedding of *E. coli* O157:H7 in cattle, including diet (8), housing (9), season (10), stress (11), and age (12). However, knowledge of the microbial ecology of the gastrointestinal tract of cattle is limited, and relationships among microbiota may impact the ability of *E. coli* O157:H7 to persist in some cattle (13).

Endemic bacteriophages (phages) are another factor which could directly impact shedding of *E. coli* O157:H7 by cattle. Phages are natural predators of bacteria and represent a significant factor in limiting bacterial populations (14). Endemic phages have been isolated from cattle and their environment, and these phages may be highly specific and effective for biocontrol of *E. coli* O157:H7 in cattle (15–17). Knowledge of *E. coli*/phage ecology *in vivo* is relatively limited, and the effects of endemic phages on the efficacy of phage cocktails used in biocontrol are unknown, making interpretation of these studies difficult (18). A wider knowledge of the natural microbiota in the cattle environment and the relationship between naturally occurring phages and *E. coli* O157:H7 would improve current mitigation methods. Accordingly, the objectives of this study were to identify endemic phages in the feedlot environment and determine the relationship of these phages to naturally occurring *E. coli* O157:H7 in cattle identified from a single fecal sample as shedding high and low numbers of *E. coli* O157:H7.

MATERIALS AND METHODS

Sample collection. The study was conducted at the Lethbridge Research Center (LRC) containment feedlot (Lethbridge, Alberta, Canada) from 8 July to 7 August 2011. All cattle were handled in accordance with the Canadian Council of Animal Care (19) with procedures approved by the LRC Animal Care Committee. Fecal grab samples from crossbred yearling steers (*n* = 400) at a commercial feedlot in southern Alberta were sampled once to identify supershedders (SS) (20). Based on this single evaluation, six cattle excreting $\geq 10^4$ CFU · g^{-1} of feces, classed as supershedders (SS), and five cattle excreting $< 10^4$ CFU · g^{-1} of feces, classed as low shedders (LS), were then transported to LRC. The six SS were a subsample of SS used in a companion study (20), while the five randomly selected LS were not part of the companion study. Cattle were penned individually for 2 weeks and grouped together for the final 3 weeks of the experiment to meet requirements of the companion study. Approximately 50 g of freshly voided fecal pats was collected from each steer daily for the first 6 days of the trial using sterile gloves and placed in 90-ml polypropylene containers (Fisher Scientific, Nepean, ON, Canada). Fecal grabs (50 g) were collected from cattle for the remainder of the trial with the exception of 5 days when no samples were collected (Fig. 1). Samples were immediately transported to the laboratory and stored at 4°C for further analysis.

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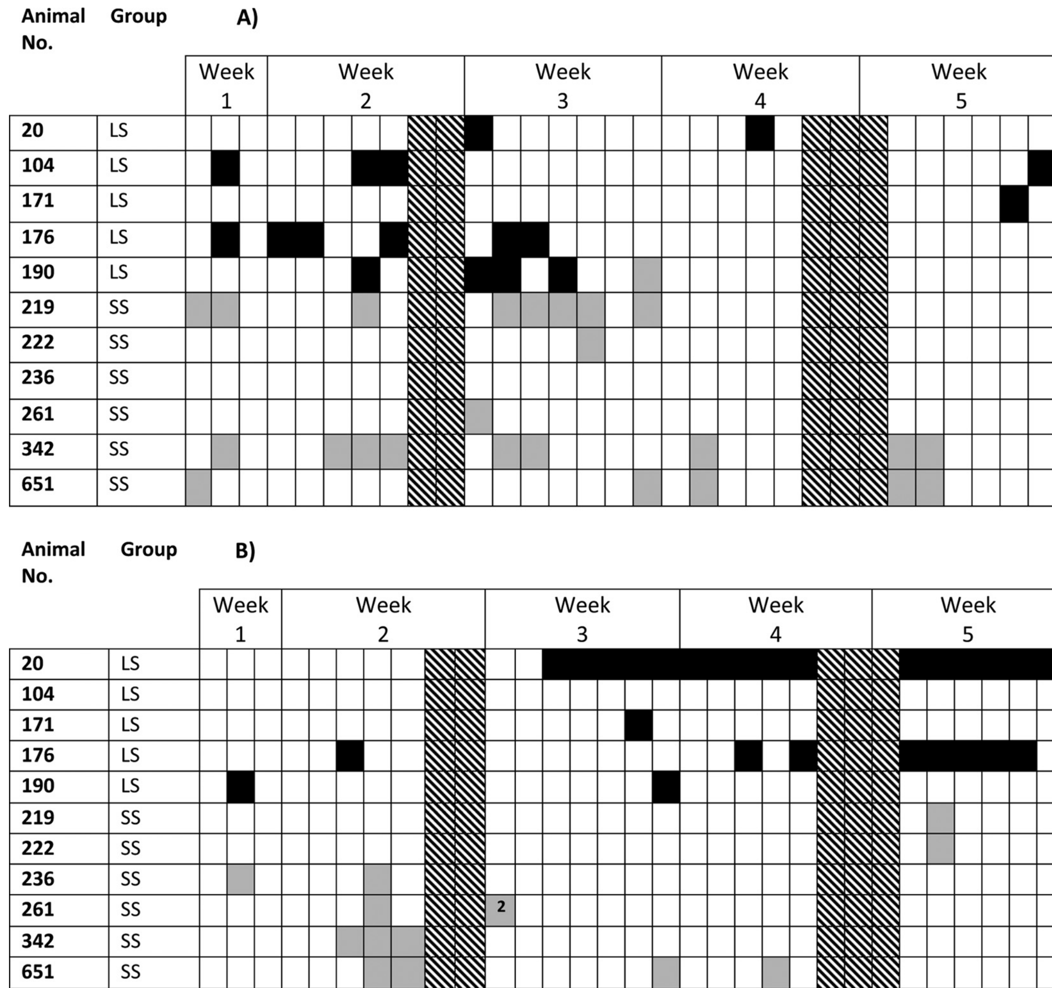

FIG 1 Presence of *E. coli* O157:H7 (A) and phages (B) in fecal samples (pats or grabs) from steers ($n = 11$) from 8 July to 7 August 2011. Black boxes represent steers designated low shedders (LS), and gray boxes represent steers designated supershedders based on a single fecal sample collected at a commercial feedlot. Hatched boxes represent days when no samples were taken. For animal 261 in week 3, two types of phages (as indicated in the box) were isolated on the designated sampling day.

Isolation and enumeration of *E. coli* O157:H7 bacteria. *E. coli* O157:H7 in fecal pats and in fecal grab samples were enumerated by direct plating on sorbitol MacConkey agar with 2.5 mg · liter^{-1} potassium tellurite and 0.05 mg · liter^{-1} cefixime (Daylynn Biologicals, Calgary, Alberta, Canada), and plates with 30 to 300 colonies were used for enumeration. Duplicate 1-g subsamples of feces were enriched in 9 ml of modified *E. coli* broth with 20 mg · liter^{-1} novobiocin and incubated for 6 h at 37°C. For detection of *E. coli* O157:H7, enriched samples were subjected to immunomagnetic separation using anti-*E. coli* O157 Dynabeads (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's instructions. Five random non-sorbitol-fermenting colonies from each plate were tested for the O157 antigen using an O157 latex agglutination kit (Oxoid, Nepean, Ontario, Canada), and numbers of *E. coli* O157:H7 bacteria were adjusted by the proportion of positive latex tests. Colonies were also confirmed positive by PCR as described previously (21). Positive *E. coli* O157:H7 isolates were frozen in glycerol and stored at −40°C.

PFGE of *E. coli* O157:H7 isolates. Frozen isolates were resuscitated on Luria-Bertani agar (EMD Chemicals, Gibbstown, NJ, USA) and incubated at 37°C overnight. Isolates were typed by pulsed-field gel electrophoresis (PFGE) of genomic DNA digested with XbaI (New England BioLabs, Pickering, Ontario, Canada) according to a standardized protocol for molecular subtyping of *E. coli* O157:H7 (22) using a CHEF DR II electrophoresis unit (Bio-Rad Laboratories, Mississauga, ON, Canada). Resulting patterns were analyzed using BioNumerics, version 6.6, software (Applied Maths, Inc., Austin, TX), with closely related isolates having at least 90% similarity.

Isolation of *E. coli* O157:H7-infecting phages. *E. coli* O157:H7 R508, a bovine phage type 14 (PT14) strain supplied by the Laboratory for Foodborne Zoonoses, Public Health Agency of Canada (PHAC), Guelph, ON, Canada, was used as the host for isolation of phages. To isolate phages from fecal samples (grabs or pats), 2 g of feces was suspended in 8 ml of lambda diluent (10 mM Tris-Cl [pH 7.5], 8 mM $MgSO_4 \cdot 7H_2O$), vortexed thoroughly, and left to stand at room temperature for 30 to 60 min. Samples were then centrifuged at 5,250 × *g* for 10 min (X-15R; Beckman Coulter, Mississauga, ON, Canada) to sediment feces. A subsample (1.8 ml) was extracted from the top layer, centrifuged at 11,000 × *g* for 10 min, and filtered through a 0.22-μm-pore-size syringe filter (Pall Life Science, Mississauga, ON, Canada). Filtrates were subjected to short (1 h) and/or long (overnight; 23 h) enrichment for detection of phages and evaluation of phage populations. Phages were purified three times by single-plaque isolation, and stock filtrates were prepared using *E. coli* O157:H7 R508 as a host as described previously (16). Titers of phages (~1×10^8 PFU · ml^{-1}) in stock filtrates were determined using the soft-agar overlay technique (24).

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Genome size estimation, RFLP, and TEM of phage isolates. Phage stocks (50 ml) were treated with DNase (10 $\mu g \cdot ml^{-1}$; Sigma-Aldrich, Oakville, ON, Canada) and RNase (20 $\mu g \cdot ml^{-1}$; Sigma-Aldrich) for 1 h at room temperature on a magnetic stirrer. Phage suspensions were then treated with 1 M NaCl and 10% polyethylene glycol (PEG) 8000 and slowly mixed overnight at 4°C. Overnight samples were centrifuged at 14,000 × *g* for 30 min (236 HK; Hermle, Edison, NJ, USA) at 4°C, and supernatants were removed. The pellet was resuspended in 2 ml of lambda diluent (pH 7.2). Genome size of concentrated phage lysates was determined by PFGE as described previously (16) using a CHEF DR II electrophoresis unit (Bio-Rad Laboratories, Mississauga, ON, Canada). Resulting band sizes were analyzed using BioNumerics. Determination of restriction fragment length polymorphism (RFLP) was performed on pretreated phage stocks using PFGE (16). Filtered phage lysates were examined by transmission electron microscopy (TEM) according to methods described previously (16).

Phage typing. *E. coli* O157:H7 isolates were serotyped and phage typed at the *E. coli* Reference Laboratory for Food-borne Zoonoses, Guelph, Ontario, Canada. Phage typing was performed as described previously (25, 26) with 16 phages that currently differentiate 89 phage types.

Microplate phage virulence assay. The susceptibility of *E. coli* O157:H7 to isolated phages was determined by a microplate phage virulence assay as described previously (16). A collection of commonly isolated PTs of *E. coli* O157:H7 ($n = 30$) supplied by PHAC were used in the assay. Six *E. coli* O157:H7 samples isolated in the current study (from each PFGE subtype) were also included in the phage assays. The multiplicity of infection (MOI) was estimated by the minimum concentration of phage required for complete lysis of the culture. Susceptibility of *E. coli* O157:H7 bacteria was defined as follows: extremely sensitive, MOI of <0.01; highly sensitive, MOI of ≥0.01 and <1; moderately sensitive, MOI of ≥1 and <10; and resistant, no lysis upon exposure to phage isolates.

Statistical analyses. Bacterial enumerations were log transformed into the number of CFU per gram of feces. Phage and *E. coli* O157:H7 prevalence data were converted to binary data, where the presence of plaques or colonies was considered positive and the absence of plaques or colonies was considered negative. Presence of phage and *E. coli* O157:H7 was analyzed using a logit link function and binomial distribution within the GLIMMIX procedure of SAS (SAS for Windows, version 9.2; SAS Institute Inc., Cary, NC, USA). Animal type (SS or LS), week of sampling (1 through 5), and interaction between animal type and week of sampling were the independent variables for detection of phage, while animal type, week of sampling, and presence of phage were independent variables for detection of *E. coli* O157:H7. In all GLIMMIX analyses, differences were considered significant at a *P* value of <0.05, and restricted maximum likelihood (REML) was used to estimate the variance component.

RESULTS

Prevalence of *E. coli* O157:H7. The total prevalence of *E. coli* O157:H7 isolated from feces of 11 steers was 41/285 (14.4%; data represent the number of positive samples/total number of samples tested). Prevalences of fecal samples positive for *E. coli* O157:H7 did not differ ($P = 0.972$) between SS (22/156, 14.1%) and LS (19/129, 14.7%) (Fig. 1) groups. Although there was no difference in the prevalences of *E. coli* O157:H7 among sampling days ($P = 0.989$) across all cattle, the prevalence of *E. coli* O157:H7 tended to vary by week. Prevalence of the pathogen (18.4 to 21.2%) was higher in the first 2 weeks of the study than in the following 2 weeks (7.6 to 9.1%; $P < 0.05$). Average concentrations of *E. coli* O157:H7 were 3.38 log CFU $\cdot g^{-1}$ feces for SS steers and 2.50 CFU $\cdot g^{-1}$ feces for LS steers. Only two SS steers and no LS had a fecal sample with ≥4.00 $\log_{10}$ CFU $\cdot g^{-1}$ of *E. coli* O157:H7 during the trial.

***E. coli* O157:H7 genotypes.** A total of 41 isolates were confirmed as *E. coli* O157:H7 by multiplex PCR. Based on PFGE patterns, positive isolates were classified into six subtypes with 90% similarity (Table 1). *E. coli* O157:H7 subtype 3 was isolated from both SS (86.4%) and LS (63.2%) steers. *E. coli* O157:H7 subtypes 1 and 6 were isolated only from SS, while subtypes 2, 4, and 5 were isolated only from LS.

TABLE 1 Number of isolates of *E. coli* O157:H7 PFGE subtypes and phage groups from feces of each steer

Steer type and no.[a]	No. of isolates								
	E. coli O157:H7 PFGE subtype ($n = 41$)[b]						Phage group[c]		
	1	2	3	4	5	6	α	β	γ
LS									
20			3					1	15
104		1	3						
171				1				1	
176			1		5		1		7
190			5				1	1	
SS									
219			7			1			1
222			1					1	
236							1	1	
261			1				2	1	
342	1		7				3		
651	1		3				3	1	

[a] Supershedders (SS) and low shedders (LS) were categorized from one fecal grab sample collected at a commercial feedlot ($n = 11$ animals).
[b] *E. coli* O157:H7 subtypes ($n = 41$) are based on ~90% similarity among isolates.
[c] Phage groups ($n = 41$) are based on genome size.

Prevalence of phages. Fecal samples positive for phages were found in both SS and LS cattle (Fig. 1B). Overall, the prevalence of phages in the SS group (13/156, 8.3%) was considerably lower ($P = 0.01$) than in the LS group (27/129, 20.9%). In SS steers, phages were detected more frequently during the first week (8/40, 16.7%) than in subsequent weeks (2.8 to 5.6%) although this difference was not significant ($P = 0.078$). In contrast, phages were more frequently isolated from LS steers during the last sampling week (36.7%), at levels higher ($P < 0.05$) than those obtained in the first 2 weeks (5.1 to 13.3%). Moreover, the prevalences of phages in the third and fourth weeks from LS animals were substantially higher ($P \leq 0.01$) than in the corresponding weeks from SS steers. All phages isolated from short (1 h) enrichments were from LS cattle, suggesting high phage populations in these steers (prevalence, 2/5).

Phage genome sizing and phage groups. A total of 41 isolated phages were divided into three groups (α, β, and γ) based on PFGE genome sizes (Table 1). The genome sizes were 42 kb for phage group α, 92 kb for phage group β, and 183 kb for phage group γ. Two types of plaque morphology were observed: small (<1-mm diameter) or bull's-eye (~1- to 2-mm diameter clearing zone with translucent halos). All isolates in groups β and γ displayed small plaques, while isolates in group α displayed the bull's-eye morphology. On one occasion, phages isolated from a single fecal sample from one SS steer exhibited both morphologies. Phage group α was most frequently isolated from SS (64.3%), while group γ was most frequently isolated from LS (81.5%). One phage from each phage group was selected for further characterization based on having a high titer against strain R508: vB_EcoS_ALC35 was selected from group α at an MOI of 1, vB_EcoM_ALC54 was selected from group β at an MOI of 0.001, and vB_EcoM_ALS20 was selected from group γ at an MOI of 0.0001.

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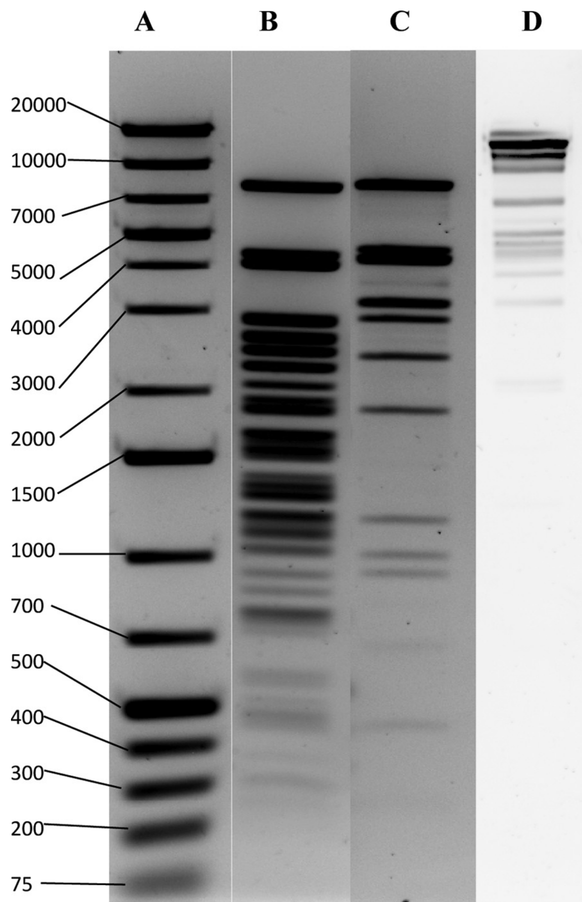

FIG 2 Restriction fragment length polymorphism (RFLP) of phage genomes. The following are represented: 1-kb Plus DNA ladder (Fermentas, Carlsbad, CA) (A), ALS20 digested by HindIII (B), ALC35 digested by HindIII (C), and ALC54 digested by EcoRV (D).

RFLP of phage groups. RFLP patterns showed multiple restriction sites for phage ALS20 (Fig. 2B) and ALC35 (Fig. 2C) by HindIII. In contrast, the ALC54 phage genome was left intact using enzymes HindIII, EcoRI, BM-O, BM-N, PstI, XbaI, KpnI, XhoI, SacI, Acc651, SalI, and AluI, although multiple restriction sites were found for this phage using enzyme EcoRV (Fig. 2D).

TEM and characterization of phages. Phages from each group displayed morphologically distinct structures with TEM. Structurally, phage ALC35 had a head diameter of 58 nm and a long noncontractile tail of 156 by 8 nm with tail fibers (Fig. 3A). Based on morphology and genome size, ALC35 was identified as a T1-like phage of the *Siphoviridae*. ALS20 had an isometric head of 72 nm in diameter and a contractile tail of 107 by 18 nm and was identified as an O1-like phage of the *Myoviridae* (Fig. 3B). ALC54 had a large elongated head and a striated contractile tail of 133 by 16 nm and was characterized as a T4-like phage of the *Myoviridae* family (Fig. 3C).

Sensitivity of *E. coli* O157:H7 to endemic phages. The *E. coli* O157:H7 host strain R508 was confirmed as highly sensitive to the phages selected from each group (ALC35, ALC54, and ALS20) (Table 2). Six strains of *E. coli* O157:H7, isolated with the phages (three from the SS and three from the LS groups) and representing the six different PFGE subtypes, were also tested against phages to determine *E. coli* O157:H7 sensitivity. The six "local" *E. coli* O157:H7 isolates were phage typed as PT4, PT14a ($n = 3$), PT43, and PT91. All 30 PTs in the strain collection and all six locally isolated *E. coli* O157:H7 strains were extremely sensitive to phage ALC54. For phage ALS20, resistance was noted for *E. coli* O157:H7 PT51 and PT91 although all other strains were extremely or highly sensitive. Lysis by phage ALC35 was variable, ranging from extremely sensitive (52.7%; 19/36) to highly sensitive (16.7%; 6/36), moderately sensitive (27.7%; 10/36), or resistant (2.78%; 1/36).

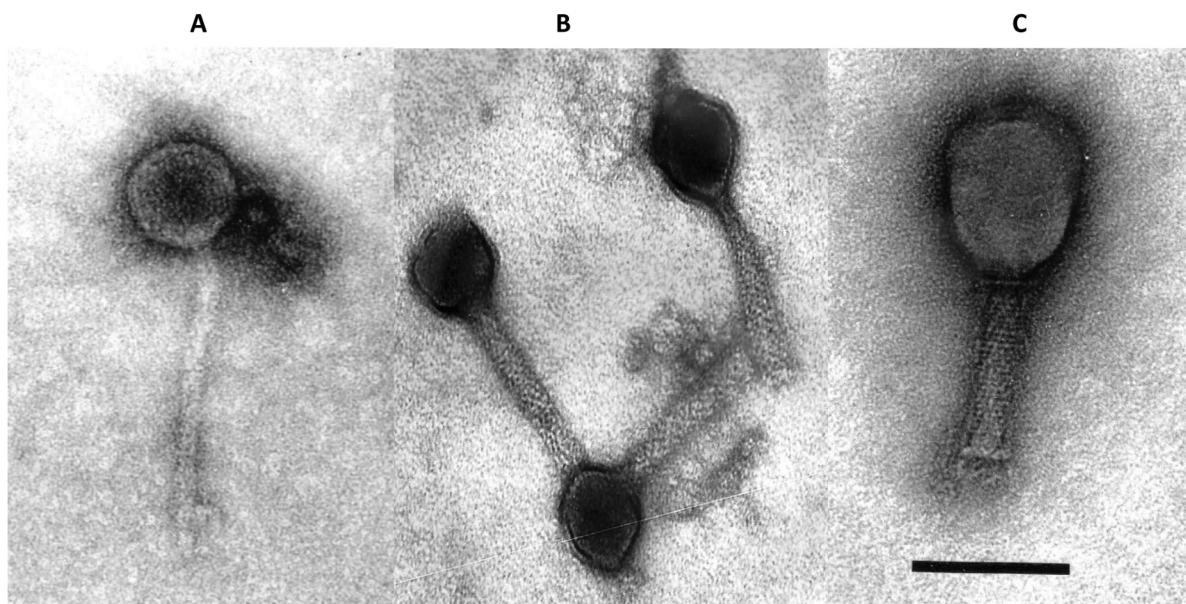

FIG 3 Transmission electron microscopy of ALC35, a T1-like phage (A), ALS20, an O1-like phage (B), and ALC54, a T4-like phage (C). Scale bar, 100 nm. Cells were negatively stained with uranyl acetate.

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TABLE 2 Susceptibility of *Escherichia coli* O157:H7 phage types to phages ALC35, ALS20 and ALC54[a]

Phage	Phage group[b]	Extremely sensitive		Highly sensitive		Moderately sensitive PTs	Resistant LPT[e]
		PTs[c]	LPTs[d]	PTs	LPT		
ALC35	α	1, 10, 14, 14a, 21, 23, 28, 32, 34, 46, 47, 48, 68, 80, 88	4, 14a	4, 24, 49, 67, 74	43	2, 8, 31, 33, 38, 45, 50, 51, 54, 63	91
ALS20	β	1, 4, 24, 28, 45, 46, 47, 49, 50, 67, 68, 74, 88	14a, 43	2, 8, 10, 14, 14a, 21, 23, 31, 32, 33, 34, 38, 48, 54, 63, 80	4		91
ALC54	γ	1, 2, 4, 8, 10, 14, 14a, 21, 23, 24, 28, 31, 32, 33, 34, 38, 45, 46, 47, 48, 49, 50, 51, 54, 63, 67, 68, 74, 80, 88	4, 14a, 43, 91				

[a] Susceptibility was defined as follows: extremely sensitive, MOI of <0.01; highly sensitive, MOI ≥0.01 and <1; moderately sensitive, MOI of ≥1 and <10; resistant, no lysis observed.
[b] Phage groups are based on genome sizes: 42.4 kb for group α, 91.6kb for group β, and 182.7 kb for group γ.
[c] PTs are phage types from *E. coli* O157:H7 collection of the Laboratory for Food-borne Zoonoses, Public Health Agency of Canada (PHAC), Guelph, ON, Canada.
[d] LPTs are phage types of *E. coli* O157:H7 isolated during the present study.
[e] There were no resistant PTs or LPTs for ALC54.

DISCUSSION

***E. coli* O157:H7 prevalence.** The overall prevalence of fecal shedding of *E. coli* O157:H7 was 14.4%, similar to results of other studies of feedlot cattle in summer months (10, 27). The prevalences of *E. coli* O157:H7 in the study in SS and LS groups were similar, suggesting that most SS steers did not remain persistent shedders after transport to the research facility. Classifying cattle with relation to shedding level was difficult as one SS did not shed *E. coli* O157:H7 for the duration of the study after relocation. Individual cattle may be supershedders only for a short period of time (28, 29), and *E. coli* O157:H7 prevalence gradually decreased over the course of the study. Although most SS did not continue to shed *E. coli* O157:H7 at SS levels, the numbers of *E. coli* O157:H7 excreted were higher in SS than in LS, possibly due to enhanced colonization of the gastrointestinal tract by *E. coli* O157:H7 in SS. Accordingly, cattle persistently shedding *E. coli* O157:H7 have been shown to have 10 to 100 times greater colonization of intestinal sites than nonpersistent shedding cattle (30).

***E. coli* O157:H7 subtypes.** The majority (75.6%) of *E. coli* O157:H7 isolated from both SS and LS cattle was from one dominant PFGE subtype as has been previously reported (10, 31) and suggests that certain dominant subtypes persist or readily disseminate among cattle. Some studies indicate that SS cattle may carry unique subtypes of *E. coli* O157:H7 (4, 32), whereas other studies do not identify common subtypes exclusive to high-shedding animals (6, 33, 34). In the current study, distinct PFGE subtypes were found exclusively in SS or LS cattle, suggesting that a relationship exists between subtypes of *E. coli* O157:H7 and the number of *E. coli* O157:H7 shed by cattle.

Phage typing revealed PFGE subtypes 2, 3, and 5 as PT14a, which has emerged as the predominant PT of *E. coli* O157:H7 isolated from human disease outbreaks in Canada (35) and suggested that both SS and LS cattle harbored a highly pathogenic strain capable of causing disease in humans. The heterogeneity of *E. coli* O157:H7 excretion by individual cattle involves colonization by the bacteria of the gastrointestinal tract. Persistently shedding cattle may be influenced by unknown factors that favor colonization by specific strains of *E. coli* O157:H7 (30). Supershedder and LS cattle are affected by seasonality (29), but relationships within intestinal microflora are still poorly understood, and endemic phages may influence clearance, proliferation, or possibly supershedding of *E. coli* O157:H7 by individual cattle.

Prevalence of phages. The overall prevalences of phages from fecal samples of SS (8.3%) and LS (20.9%) were similar to the range (11.7 to 23.3%) reported previously (36). Phages may fluctuate in concert with populations of *E. coli* O157:H7 (23), and low populations of *E. coli* O157:H7 may have been due to the presence of O157-specific phages in the present study. Low-shedding cattle had higher concentrations of phages and lower numbers of *E. coli* O157:H7 bacteria than SS, suggesting that the presence of phages may influence shedding.

Characterization of endemic phages. Based on genome size and TEM morphology, the phage isolates were classified into T1-like phage of *Siphoviridae* and O1-like and T4-like phages of *Myoviridae*.

ALC35: T1-like phage. Other endemic T1 phages isolated from the feedlot environment have had genome sizes of ~44 kb and plaques with wide opalescent zones, while restriction digestion analysis indicated 8 to 12 fragments after digestion with HindIII, similar to phage ALC35 (16, 18). Transmission electron microscopy revealed a general resemblance to phage T1 (16). ALC35 displayed highly variable lysis patterns on *E. coli* O157:H7 isolates, indicating that the lytic capability of this phage probably varied with the host. *Escherichia coli* PTs 2 and 8, which are commonly isolated from outbreaks in nursing homes (37) and elsewhere in Canada (35), were only moderately sensitive to ALC35.

ALS20: O1-like phage. The genome size of ALS20 from phage group β was 91.6 kb and very close to that of Felix O1 (88.9 kb) and the O1-like wV8 virus (86.1 kb) (38). Restriction analysis of ALS20 showed multiple cleavage sites by HindIII, but restriction sites differed from those of the O1 virus, FO1, where two cleavage sites were found after HindIII digestion (39). Most *E. coli* O157:H7 PTs were extremely or highly sensitive to ALS20 although resistance to PT51 and the locally isolated PT91 was observed. PT51 and PT91 are not commonly isolated from humans in Canada (35). In addition to *E. coli* O157:H7, the Felix O1-like virus and phage wV8 virus are able to lyse many common *Salmonella* strains (38), suggesting that O1-like phages may make good candidates for phage therapy by targeting multiple pathogenic bacterial species capable of causing disease in humans.

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ALC54: T4-like phage. The genome size of ~182.7 kb of ALC54 was similar to that of several T4 phages specific for *E. coli* O157:H7 that have been isolated with genome sizes ranging from 166 to 180 kb (40–42). Restriction endonuclease digestion of ALC54 determined that most enzymes were unable to cut the genome, which is similar to findings with other previously isolated *E. coli* O157-specific T4 phages (42, 43). Electron microscopy of ALC54 revealed myovirus morphology and a large head like that of other T4-like phages (41, 42). Most endonucleases do not digest T4 DNA due to the presence of glycosylated hydroxylmethyl cytosine instead of cytosine (44). All *E. coli* O157:H7 PTs and locally isolated *E. coli* O157:H7 strains were extremely sensitive to lysis by ALC54, suggesting that this T4-like phage is an efficient predator of *E. coli* O157:H7. T4-like phages isolated from dairy and beef feedlot manure lysed 94 to 98% of *E. coli* O157:H7 as determined previously (45), and although their lytic capabilities varied among isolates, an eight-phage cocktail was capable of reducing *E. coli* O157:H7 populations by $>5 \log \text{CFU} \cdot \text{ml}^{-1}$ *in vitro* at 37°C. The broad host range of T4-like phages stems from their ability to recognize several host lipopolysaccharides, outer membrane proteins, and C termini specific for adsorption of *E. coli* O157:H7, making T4 phages excellent candidates for phage biocontrol (42, 43). Phages are widespread in livestock (23), and the current study suggests that T4-like endemic phages are persistent in some cattle.

Conclusions. This study suggests a role for endemic phages in controlling naturally occurring *E. coli* O157:H7. Most steers harbored *E. coli* O157:H7 PT 14a, which is capable of causing severe disease in humans. Compared to T1- and O1-like phages, T4-like phages exhibited a broader host range and stronger lytic capability when targeting *E. coli* O157:H7. Moreover, the T4-like phages were more frequently isolated from feces of LS than from SS, suggesting that endemic phages may play an important role in mitigating *E. coli* O157:H7 shedding in cattle and that the different shedding level of the pathogen in each animal might be partially dependent on phages. Some of the challenges facing recent phage therapy attempts are successful replication of phages and selection of phages. The impact of endemic phages on optimum phage/host concentrations and/or competition or synergy among phages should be considered when phages are assessed for biocontrol. A better understanding of the microbial ecology of the gastrointestinal tract of cattle is critical for successful management of *E. coli* O157:H7.

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