

Isolation and Characterization of a New T-Even Bacteriophage, CEV1, and Determination of Its Potential To Reduce *Escherichia coli* O157:H7 Levels in Sheep

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Bacteriophage CEV1 was isolated from sheep resistant to *Escherichia coli* O157:H7 colonization. In vitro, CEV1 efficiently infected *E. coli* O157:H7 grown both aerobically and anaerobically. In vivo, sheep receiving a single oral dose of CEV1 showed a 2-log-unit reduction in intestinal *E. coli* O157:H7 levels within 2 days compared to levels in the controls.

Escherichia coli O157:H7 is a highly virulent food-borne pathogen with an infective dose of ~100 cells in humans (5, 25). Infections can be severe; symptoms include hemorrhagic colitis, hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura, and even death (23–25, 31). Although *E. coli* O157:H7 infections can be linked to a variety of sources, primary contamination is usually ruminant related; cattle and other ruminants act as asymptomatic reservoirs (2, 6, 7, 11, 12, 15, 17, 30). Despite extensive intervention strategies, human *E. coli* O157:H7 infections still occur (7, 9, 14). Thus, recent research has focused on the development of preharvest interventions to reduce the entry of the pathogen into the food chain. Bacteriophages have been used successfully as antibacterial agents in both human and veterinary medicine and are

one potential preharvest *E. coli* O157:H7 control strategy (4, 8, 18, 20, 22, 28, 32–34, 39). In this study we describe a new T4-like bacteriophage, CEV1, isolated from sheep resistant to colonization by *E. coli* O157:H7, and we discuss its potential to control this pathogen.

Isolation and characterization of bacteriophage CEV1. Crossbred ewes ($n = 22$) were orally inoculated with ~10⁹ CFU/sheep of novobiocin-resistant *E. coli* O157:H7 (NCTC 12900). In the first 2 days following inoculation, these sheep fecally shed ~10² CFU/g 12900. By day 3, no viable 12900 was detected. Each sheep was rechallenged with ~10¹¹ CFU 12900 and monitored, with similar results. The sheep were then challenged with *E. coli* O157:H7 EDL 933 (10⁹ CFU/sheep). Again, fecal shedding decreased rapidly, and by day 4, EDL

TABLE 1. Host range of bacteriophage CEV1, as determined by spot tests^a

Type of bacteria	CEV1-sensitive strains	CEV1-resistant strains
Pathogenic	<i>E. coli</i> O157:H7: EDL 933 (ATCC 43895) ^b ; FDIU strains 86-24, 2027, 2028, 2029, 2030, 2031, 2255, 2257, 2266, 2309, 2317, 2321, 2324, 6058 <i>E. coli</i> O43:H7 <i>E. coli</i> O126:H7 <i>E. coli</i> O153:H7 <i>E. coli</i> O158:H7 <i>E. coli</i> Omulti:H7	<i>E. coli</i> O157:H7: FDIU strains 207 and 2336 <i>E. coli</i> O15:H7 <i>E. coli</i> O15:H25 <i>E. coli</i> O50:H7 <i>Salmonella enterica</i> serovar Newport <i>Salmonella enterica</i> serovar Dublin <i>Salmonella enterica</i> serovar Derby <i>Salmonella enterica</i> serovar Enteritidis <i>Salmonella enterica</i> serovar Typhimurium
Nonpathogenic	<i>E. coli</i> O157:H7: NCTC 12900 (ATCC 700728) ^c ; 87-23 ^c ECOR: 4, 8, 11, 13, 14, 16, 33, 48, 56 <i>E. coli</i> K-12, B	ECOR: 1 to 3; 5 to 7, 9, 10, 12, 15, 17 to 32, 34 to 47, 49 to 55, 57 to 72

^a The efficiencies of plating for all phage-sensitive strains were generally similar to those on B and K12; none differed by more than 1 order of magnitude.

^b Toxicogenic *E. coli* O157:H7 strain used throughout this study both in vitro and in vivo.

^c Nontoxicogenic biosafety level-1 *E. coli* O157:H7 strains used throughout this study both in vitro and in vivo. These strains are serologically and physiologically wild-type O157:H7 strains but have the pathogenicity island prophage deleted (Stx⁻).

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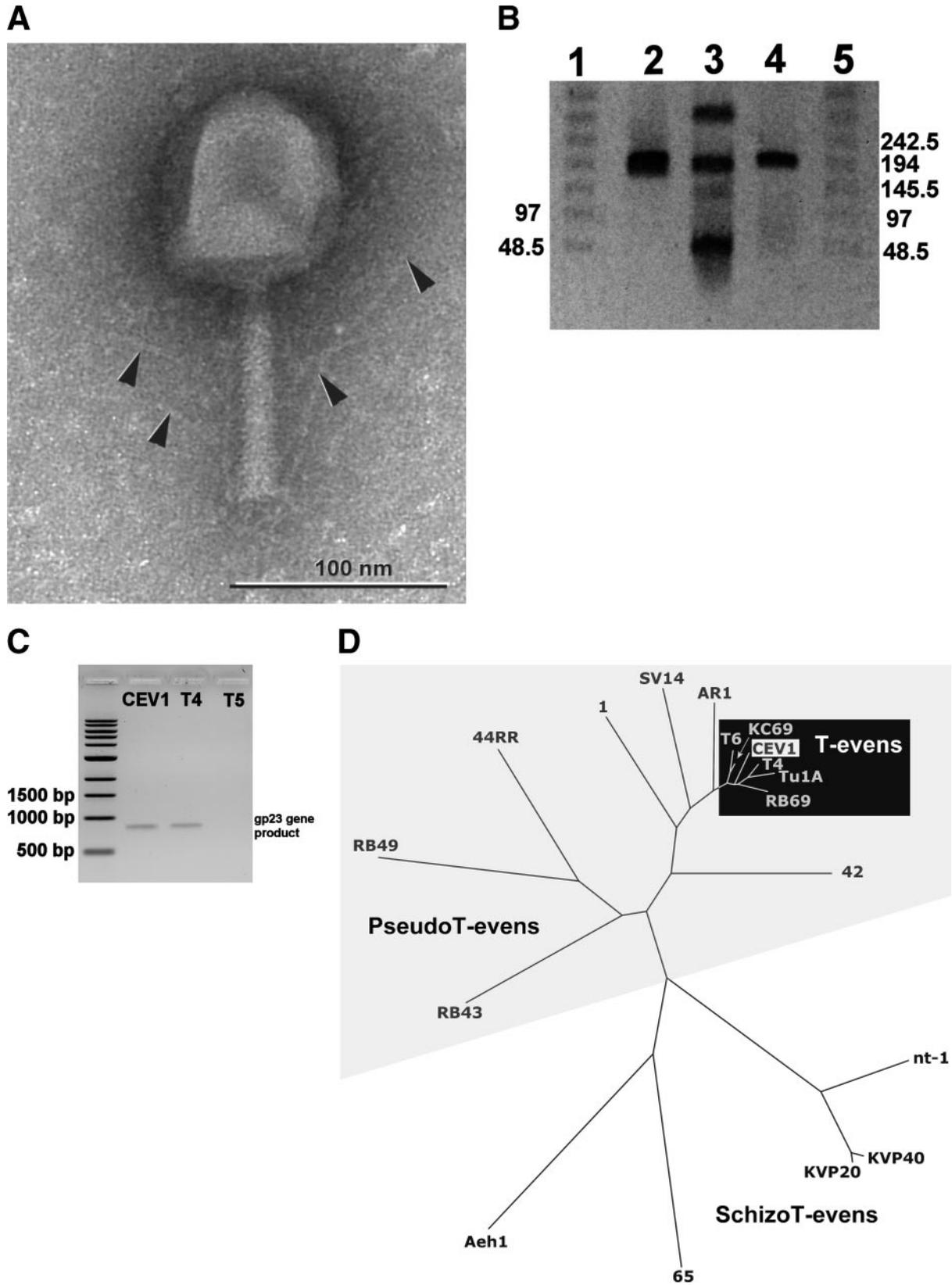


FIG. 1. Characterization of phage CEV1. (A) Electron micrograph of bacteriophage CEV1. Arrows indicate tail fibers. Dimensions: head, 100 nm by 85 nm; contractile tail, 100 nm by 15 nm. (B) Pulsed-field gel electrophoresis of phage DNA, as observed on an ethidium bromide-stained low-melting-point agarose gel (1%, wt/vol) (0.5× TBE, consisting of 45 mM Tris-borate and 1 mM EDTA [pH 8]) run for 20 h at 6 V/cm and 14°C with a pulse period of 45 to 90 s. Lanes 1 and 5, λ DNA ladder (Bio-Rad); lane 2, T4 DNA (175 kb); lane 3, DNA ladder composed of phage (*Pseudomonas*

933 levels were below the detection limit. These results were unprecedented at our facility, where both strains are routinely used *in vivo* (typically $\sim 10^4$ CFU/g is shed fecally for ~ 10 days). Feces collected from these ewes tested positive for *E. coli* O157:H7-infecting phage. By using our standard enrichment protocol, a new bacteriophage, CEV1, was isolated (10).

CEV1 was active against 17/19 *E. coli* O157:H7 strains tested, 9 strains from 4/5 phylogenetic groups of the *E. coli* reference (ECOR) collection, lab strains B and K-12, and strains from serogroups O43, O126, O153, O158, and Omulti (Table 1) (27). No representatives from serogroups O15 and O50 were lysed by CEV1. Using a set of isogenic *E. coli* K-12 mutant pairs, with one member of each pair mutant for a specific outer membrane protein, it was found that CEV1 uses *ompA* as its receptor (data not shown) (16).

Electron micrographs (Fig. 1A) showed that CEV1 has an icosahedral head, linked by a connector to a contractile tail with fibers at the distal end. The CEV1 genome of ~ 180 kb (Fig. 1B) is digested by EcoRV and NdeI but not by EcoRI, BamHI, PstI, Sall, or HindIII, suggesting that CEV1 DNA contains a modified base, probably 5-hydroxymethyl cytosine (data not shown). Based on its morphology and the sequence of a 788-nucleotide DNA fragment (GenBank accession no. AY331985) internal to the major capsid protein *gp23* (Fig. 1C), CEV1 is closely related to T4 (95% sequence similarity) (Fig. 1D) (40).

Infection experiments. Both aerobic and anaerobic CEV1 infections of 12900 were productive. Aerobic infections of 12900 (optical density at 600 nm [OD₆₀₀], ~ 0.3) were carried out in shake flasks containing tryptic soy broth (TSB) at 37°C with agitation. CEV1 displayed an eclipse period of 18 min, a latent period of 26 min, and a burst size of ~ 150 PFU/cell in experiments at a low multiplicity of infection (MOI; ~ 0.1) (data not shown). At an MOI of ~ 5 , $>99\%$ of the phages adsorbed, the bacterial population was reduced 5 log units, and progeny phages were observed within 20 min (upon treatment with CHCl₃, the burst size was ~ 100 PFU/cell) (Fig. 2A). Limited and variable lysis inhibition was observed; lysis began between 30 to 120 min, depending on conditions and the MOI, and took 30 to 60 min to reach completion. Anaerobic infections of 12900 were conducted using adaptations of the Hungate technique (N₂ headspace) in 126-ml butyl rubber-sealed serum bottles containing TSB. Within 5 min, $>97\%$ of the phages adsorbed, and by 20 min, levels of bacterial survivors fell 4 log units (Fig. 2B). Similarly efficient anaerobic infections were observed in bovine rumen fluid and colon-simulating media (data not shown) (26).

Animal studies. Animal studies were carried out humanely (Southern Plains Agricultural Research Center Institutional

Animal Care and Use Committee protocol 01-002). Prior to experimental treatment, 39 crossbred ewes from one flock were screened for O157:H7-infecting phages by using standard enrichment techniques; 19 tested negative (10). The others all contained the same new O157:H7-infecting phage (termed CEV2 and currently undergoing characterization). Sheep ($n = 8$) that tested negative were inoculated (by oral gavage) with $\sim 10^{10}$ CFU of novobiocin/nalidixic acid-resistant 933; after 3 days, 4/8 sheep were treated with a single oral dose of CEV1 ($\sim 10^{11}$ PFU). The animals were humanely euthanized 2 days later; bacterial counts (by plating on MacConkey agar containing 20 μ g/ml novobiocin and 25 μ g/ml nalidixic acid) of ruminal, cecal, and rectal contents showed that 933 levels were reduced 2 to 3 log units ($P > 0.05$) in the ceca and rectums of CEV1-treated sheep compared to levels in controls (Fig. 3).

Over the years, bacteriophages have frequently been isolated from the intestinal tracts of ruminants (1, 19, 29). Swain et al. (1996) studied bovine ruminal phage and concluded that phage help maintain microbial diversity and balance (37). Here we suggest that phage also play a role in controlling the microbial ecology in the colons of livestock—a role that could be adapted as a preharvest treatment to remove *E. coli* O157:H7 from sheep and other ruminants. In this study, we found two sheep flocks from different regions that harbored O157:H7-infecting phage (group 1 was the CEV1 isolation flock, while group 2 was the CEV1 treatment test flock [20/39 sheep harbored CEV2]). CEV1 was isolated from sheep that cleared *E. coli* O157:H7 rapidly and were therefore “resistant” to colonization. In view of the origin of CEV1 and its demonstrated *in vitro* and *in vivo* activity against these strains, its presence in these sheep is a logical explanation for these atypical results. These data emphasize the importance of screening for phage by enrichment prior to *in vivo* control/eradication studies.

Extensive characterization of candidate phages is needed before any widespread application is implemented. Host selectivity is key; CEV1 lyses most *E. coli* O157:H7 strains and a limited number of commensal *E. coli* strains. This will create minimal disturbance to the gut biota, allowing the simultaneous use of probiotic *E. coli* to replace O157:H7 within the gastrointestinal niche and/or to maintain sufficient CEV1 levels to clear very low levels of *E. coli* O157:H7.

Our finding that CEV1 is a close relative of T4 is advantageous; T4-like phages have a long history of safe application and have been intensively studied for >50 years (13, 21, 35, 36). They are exclusively lytic, and none encode pathogenicity islands, virulence/antibiotic resistance genes, or integrases. The ability of CEV1 to efficiently infect *E. coli* O157:H7 is, however, unusual among T4-like phages; of the ~ 100 such phages from our collection, only one infects *E. coli* O157:H7 at an efficiency of >0.001 (data not

φ2a [290 kb], T4 [175 kb], CEV2 [120 kb], and *Pseudomonas* φ2b [45 kb]) DNA; lane 4, CEV1 DNA. (C) Ethidium bromide-stained agarose (1%, wt/vol) gel of DNA-PCR amplification reactions using primers Cap8 (TGAAGTTACCTTCACCACGACCGG) and Mzia1 (TGTTATIGGTATGGTICGICGTGCTAT) for *gp23* (40). First lane, 1-kb DNA ladder (Invitrogen, CA); second lane, phage CEV1; third lane, phage T4 (positive control); fourth lane, phage T5 (negative control). (D) Unrooted phylogenetic tree constructed using the more variable internal region (amino acids 115 to 302) of the *gp23* head protein of a number of T4-like phages, including T4 (Protein Data Bank accession no. NP049787), T6 (Q38055), KC69 (AAF61696), Tu1a (AAF61691), SV14 (CAB01542), RB69 (AAF61699), RB49 (CAB01539), RB43 (AAF61698), and AR1 (Q9ZXI0); *Enterobacter cloacae* phage 1 (AAF61700); *Aeromonas salmonicida* phages 44RR2.8t (44RR; AAF61693) and 65 (AAF61694); *Aeromonas hydrophila* phage Aeh1 (AAF61695); *Burkholderia cepacia* phage 42 (AAF61692); *Vibrio natriegens* phage nt-1 (AAF61697); and *Vibrio parahaemolyticus* phages KVP20 (BAA25880) and KVP40 (BAA25567). Subgroups of phage types are indicated by shading: white, schizo T-evens; light gray, pseudo T-evens; black, T-evens.

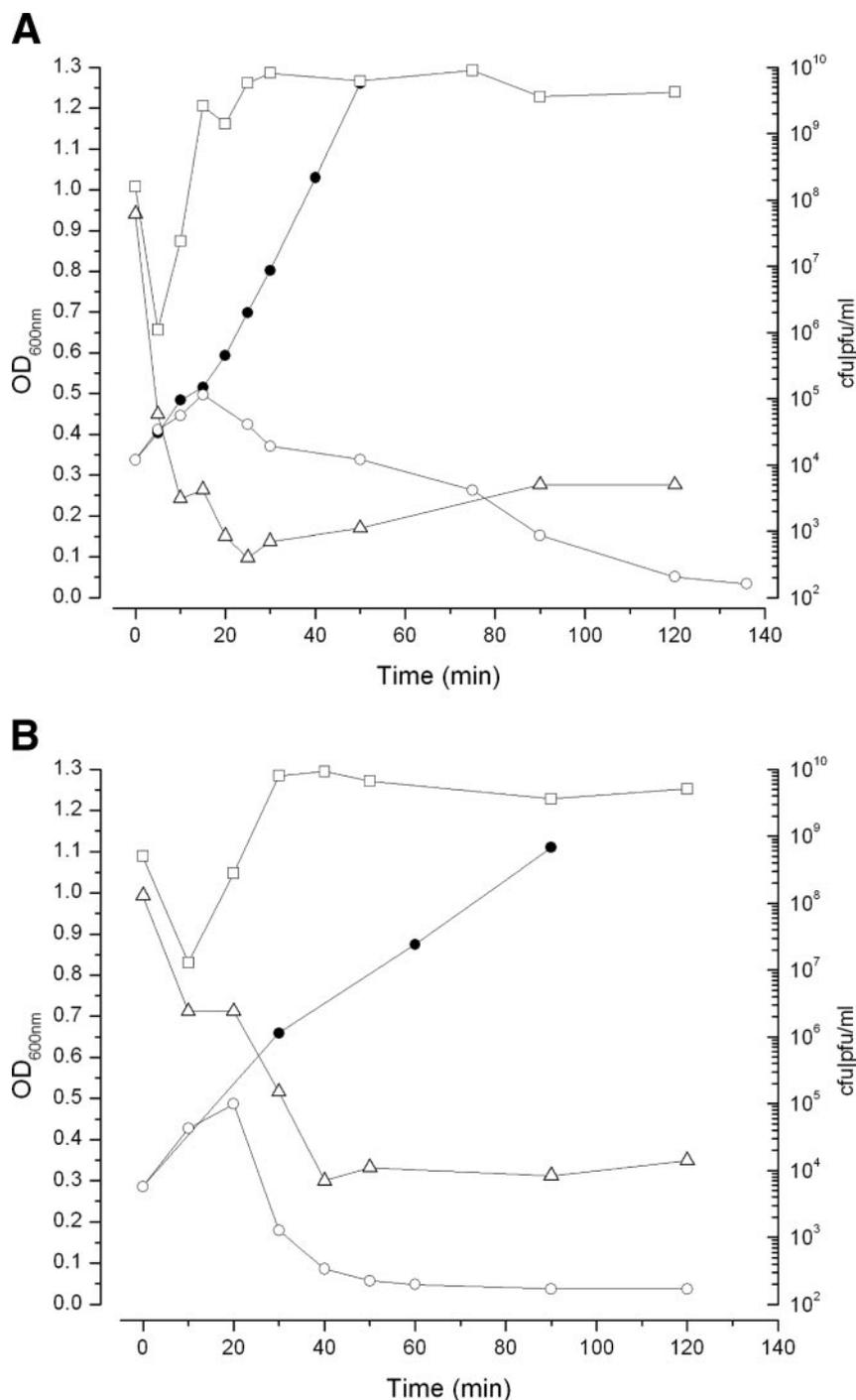


FIG. 2. CEV1 infections of *E. coli* O157:H7 NCTC 12900 growing in TSB under conditions of either aerobic respiration (A) or fermentation (B). Infection graphs shown are representative of at least three replicates. Symbols: ●, OD₆₀₀ of control cultures; ○, OD₆₀₀ of infected cultures; △, CFU of bacterial survivors/ml; □, PFU of phage/ml after the addition of chloroform.

shown). Two other reported T4-like O157:H7-infecting phages (AR1 and PPO1) do not infect lab strains B and K-12, unlike CEV1 (AR1 infects 38/72 ECOR strains, as well as some *Proteus*, *Shigella*, and *Salmonella* strains; PPO1 infects 8/72 ECOR strains, but with a specificity pattern different from that of CEV1 (data not shown) (16).

Previous researchers have discussed the applications of

O157:H7-infecting phages, but each had some physiological constraint that limited its application. O'Flynn et al. (2004) used a phage cocktail (PPO1, e4/1c, and e11/2) to eliminate *E. coli* O157:H7 from artificially contaminated meat (28). This postslaughter approach would initially appear promising in reducing the postplant *E. coli* O157:H7 load, but this application appears impractical (an MOI of $\sim 10^6$ and a temperature

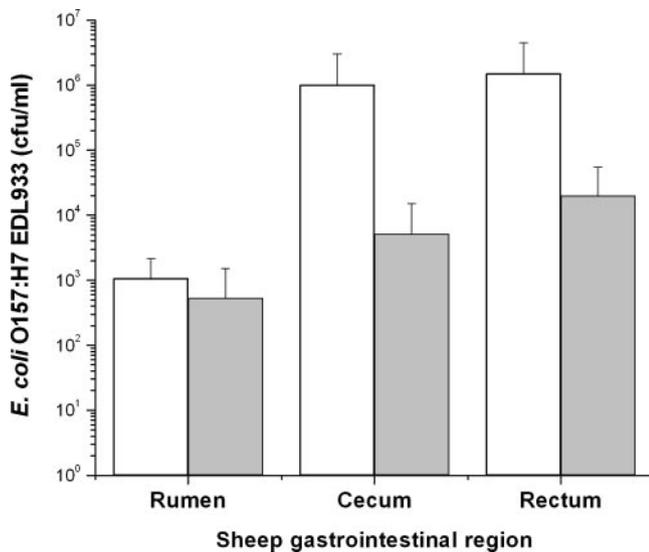


FIG. 3. Use of CEV1 as a preslaughter treatment to remove resident *E. coli* O157:H7 from the intestines of ruminants (sheep). Control sheep (white bars) received only *E. coli* O157:H7 orally on day zero, while experimental sheep (gray bars) orally received *E. coli* O157:H7 on day zero and then CEV1 on day 3. Data were collected postmortem from various parts of the animals' digestive tracts 5 days after O157:H7 inoculation and 2 days after phage treatment. Error bars, standard deviations.

of 37°C were used; no phage infection occurred at lower temperatures). In contrast, our suggested approach with CEV1 to treat the live animal and thus reduce *E. coli* O157:H7 populations before entry into the food chain, allowing the use of phage in conjunction with other methods to create a multilevel barrier.

Other preharvest *E. coli* O157:H7 phage studies have failed to demonstrate efficacy under real-world conditions. For example, Kudva et al. reported in 1999 that their O157:H7-infecting phages effectively killed EDL 932, but only aerobically (MOI, ~10³); thus, these phage were inappropriate for use in the anaerobic gut (20). Bach et al. (2003) isolated phage DC22, which infected all 23 O157:H7 strains tested in the lab but showed little or no efficacy in ruminant gut-simulating chemostats or in lambs (3). This reinforces the importance of understanding infection parameters in vitro under conditions relevant to the ecosystem where the phage will be used. Tanji et al. (2005) found that a three-phage cocktail worked effectively in vitro (aerobically and anaerobically) but was unable to clear mice of *E. coli* during in vivo studies, again indicating the complexity of this problem (38). In our study, infection of 12900 by CEV1 was productive in TSB aerobically and anaerobically, as well as in both defined and gut-simulating media (data not shown). Further, our in vivo trials show a substantial reduction in intestinal *E. coli* O157:H7 levels in sheep treated with a single oral dose of CEV1, suggesting that CEV1 shows promise as a component in a treatment for the selective reduction of *E. coli* O157:H7 levels in food animals.

In summary, although the United States is considered to have one of the world's safest food supply systems, people still become ill from bacterially contaminated food. Our results indicate that phages can reduce intestinal populations of *E. coli*

O157:H7 and could play an important role in future preharvest pathogen reduction strategies. Furthermore, in studies attempting to use intestinal ecological modification, the potential impact of naturally resident phage must not be overlooked.

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REFERENCES

- Adams, J. C., J. A. Gazaway, M. D. Brailsford, P. A. Hartman, and N. L. Jacobson. 1966. Isolation of bacteriophages from the bovine rumen. *Experientia* **22**:717-718.
- Armstrong, G. L., J. Hollingsworth, and J. G. Morris, Jr. 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.* **18**:29-51.
- Bach, S. A., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley. 2003. Effect of bacteriophage DC22 on *Escherichia coli* O157:H7 in an artificial rumen system (Rusitec) and inoculated sheep. *Anim. Res.* **52**:89-101.
- Barrow, P., M. Lovell, and A. Berchieri. 1998. Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. *Clin. Diagn. Lab. Immunol.* **5**:294-298.
- Besser, R. E., P. M. Griffin, and L. Slutsker. 1999. *Escherichia coli* O157:H7 gastroenteritis and the hemolytic uremic syndrome: an emerging infectious disease. *Annu. Rev. Med.* **50**:355-367.
- Bielaszewska, M., H. Schmidt, A. Liesegang, R. Prager, W. Rabsch, H. Tschape, A. Cizek, J. Janda, K. Blahova, and H. Karch. 2000. Cattle can be a reservoir of sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H⁻ strains and a source of human diseases. *J. Clin. Microbiol.* **38**:3470-3473.
- Brabban, A. D., D. A. Nelson, E. Kutter, T. S. Edrington, and T. R. Callaway. 2004. Approaches to controlling *Escherichia coli* O157:H7, a food-borne pathogen and an emerging environmental hazard. *Environ. Pract.* **6**:208-229.
- Callaway, T. R., R. C. Anderson, T. S. Edrington, K. J. Genovese, K. M. Bischoff, T. L. Poole, Y. S. Jung, R. B. Harvey, and D. J. Nisbet. 2004. What are we doing about *Escherichia coli* O157:H7 in cattle? *J. Anim. Sci.* **82**:E93-E99.
- Callaway, T. R., R. O. Elder, J. E. Keen, R. C. Anderson, and D. J. Nisbet. 2003. Forage feeding to reduce preharvest *Escherichia coli* populations in cattle: a review. *J. Dairy Sci.* **86**:852-860.
- Carlson, K. 2005. Appendix. Working with bacteriophages: common techniques and methodological approaches, p. 437-494. In E. Kutter and A. Sulakvelidze (ed.), *Bacteriophages: biology and applications*. CRC Press, Boca Raton, Fla.
- CDC. 2000. Summary of outbreaks of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli* reported to the CDC in 1999. [Online.] http://www.cdc.gov/NCIDOD/DBMD/diseaseinfo/files/ecoli_99summary.pdf.
- Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox, and E. Crick. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.* **111**:439-447.
- Chibani-Chennoufi, S., A. Bruttin, M. L. Dillmann, and H. Brussow. 2004. Phage-host interaction: an ecological perspective. *J. Bacteriol.* **186**:3677-3686.
- Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaria, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* **97**:2999-3003.
- Fischer, J. R., T. Zhao, M. P. Doyle, M. R. Goldberg, C. A. Brown, C. T. Sewell, D. M. Kavanaugh, and C. D. Bauman. 2001. Experimental and field studies of *Escherichia coli* O157:H7 in white-tailed deer. *Appl. Environ. Microbiol.* **67**:1218-1224.
- Goodridge, L., A. Gallaccio, and M. W. Griffiths. 2003. Morphological, host range, and genetic characterization of two coliphages. *Appl. Environ. Microbiol.* **69**:5364-5371.
- Grauke, L. J., I. T. Kudva, J. W. Yoon, C. W. Hunt, C. J. Williams, and C. J.

- Hovde. 2002. Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. *Appl. Environ. Microbiol.* **68**:2269–2277.
18. Greer, G. G. 2005. Bacteriophage control of foodborne bacteria. *J. Food Prot.* **68**:1102–1111.
 19. Klieve, A. V., and T. Bauchop. 1988. Morphological diversity of ruminal bacteriophages from sheep and cattle. *Appl. Environ. Microbiol.* **54**:1637–1641.
 20. Kudva, I. T., S. Jelacic, P. I. Tarr, P. Youderian, and C. J. Hovde. 1999. Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. *Appl. Environ. Microbiol.* **65**:3767–3773.
 21. Kutter, E., K. Gachechiladze, A. Poglazov, E. Marusich, M. Shneider, P. Aronsson, A. Napuli, D. Porter, and V. Mesyanzhinov. 1995. Evolution of T4-related phages. *Virus Genes* **11**:285–297.
 22. Loc Carrillo, C., R. J. Atterbury, A. El-Shibiny, P. L. Connerton, E. Dillon, A. Scott, and I. F. Connerton. 2005. Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Appl. Environ. Microbiol.* **71**:6554–6563.
 23. Mead, P. S., and P. M. Griffin. 1998. *Escherichia coli* O157: H7. *Lancet* **352**:1207–1212.
 24. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* **5**:607–625.
 25. Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* **11**:142–201.
 26. Nisbet, D. J., D. E. Corrier, C. M. Scanlan, A. G. Hollister, R. C. Beier, and J. R. DeLoach. 1993. Effect of a defined continuous-flow derived bacterial culture and dietary lactose on *Salmonella typhimurium* colonization in broiler chickens. *Avian Dis.* **37**:1017–1025.
 27. Ochman, H., and R. K. Selander. 1984. Standard reference strains of *Escherichia coli* from natural populations. *J. Bacteriol.* **157**:690–693.
 28. O'Flynn, G., R. P. Ross, G. F. Fitzgerald, and A. Coffey. 2004. Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* **70**:3417–3424.
 29. Orpin, C. G., and E. A. Munn. 1973. The occurrence of bacteriophages in the rumen and their influence on rumen bacterial populations. *Experientia* **30**:1018–1020.
 30. Rasmussen, M. A., W. C. Cray, T. A. Casey, and S. C. Whipp. 1993. Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli*. *FEMS Microbiol. Lett.* **114**:79–84.
 31. Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* **308**:681–685.
 32. Smith, H. W., and M. B. Huggins. 1983. Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *J. Gen. Microbiol.* **129**:2659–2675.
 33. Smith, H. W., M. B. Huggins, and K. M. Shaw. 1987. The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. *J. Gen. Microbiol.* **133**:1111–1126.
 34. Smith, H. W., M. B. Huggins, and K. M. Shaw. 1987. Factors influencing the survival and multiplication of bacteriophages in calves and in their environment. *J. Gen. Microbiol.* **133**:1127–1135.
 35. Sulakvelidze, A., and P. Barrow. 2005. Phage therapy in animals and agribusiness, p. 335–380. *In* E. Kutter and A. Sulakvelidze (ed.), *Bacteriophages: biology and applications*. CRC Press, Boca Raton, Fla.
 36. Sulakvelidze, A., and E. Kutter. 2005. Bacteriophage therapy in humans, p. 381–436. *In* E. Kutter and A. Sulakvelidze (ed.), *Bacteriophages: biology and applications*. CRC Press, Boca Raton, Fla.
 37. Swain, R. A., J. V. Nolan, and A. V. Klieve. 1996. Natural variability and diurnal fluctuations within the bacteriophage population of the rumen. *Appl. Environ. Microbiol.* **62**:994–997.
 38. Tanji, Y., T. Shimada, H. Fukudomi, K. Miyanaga, Y. Nakai, and H. Unno. 2005. Therapeutic use of phage cocktail for controlling *Escherichia coli* O157:H7 in gastrointestinal tract of mice. *J. Biosci. Bioeng.* **100**:280–287.
 39. Tanji, Y., T. Shimada, M. Yoichi, K. Miyanaga, K. Hori, and H. Unno. 2004. Toward rational control of *Escherichia coli* O157:H7 by a phage cocktail. *Appl. Microbiol. Biotechnol.* **64**:270–274.
 40. Tetart, F., C. Desplats, M. Kutateladze, C. Monod, H.-W. Ackermann, and H. M. Krisch. 2001. Phylogeny of the major head and tail genes of the wide-ranging T4-type bacteriophages. *J. Bacteriol.* **183**:358–366.