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Are Super-Shedder Feedlot Cattle Really Super?

Krysty D. Munns,[1,2] Lorna Selinger,[1] Kim Stanford,[3] L. Brent Selinger,[2] and Tim A. McAllister[1]

Abstract

The objective of this study was to determine the frequency and duration of super-shedding in cattle by enumerating *Escherichia coli* O157:H7 in feces and to compare lineage and pulsed-field gel electrophoresis (PFGE) subtypes from super- and low-shedders. *E. coli* O157:H7 was enumerated from fecal samples obtained from the rectums of 400 feedlot cattle. Super-shedding steers ($N = 11$) were identified, transported, and penned individually. Freshly voided fecal pats were sampled 2 h before and 6 h after feeding for 7 d, then once daily for an additional 19 d. Isolates ($N = 126$) were subtyped using PFGE, and lineage was typed using a lineage-specific polymorphism assay. Of the 11 super-shedders identified at the commercial feedlot, only five were confirmed as super-shedders at the research feedlot, with no super-shedders identified 6 d after sampling at the commercial feedlot. Super-shedding was not consistent in fecal pats collected from the same individual at different times of the day. Isolates exhibited three distinct PFGE subtypes, with most isolates (97.6%) displaying the same subtype, including those obtained from steers that transitioned from super- to low-shedding. The short duration of super-shedding and its lack of continuance suggest that these individuals may not play as great a role in the dissemination of *E. coli* O157:H7 within the feedlot as previously proposed.

Introduction

Healthy cattle transiently host *Escherichia coli* O157:H7 and directly or indirectly transmit this major foodborne pathogen to humans (Rangel *et al*., 2005). The load and frequency of *E. coli* O157:H7 shedding varies greatly among individual cattle (Stanford *et al*., 2005). Studies typically report shedding of the organism to be sporadic and of short duration, ranging from 10 to 10^7 colony-forming units (CFU)/g feces (Chase-Topping *et al*., 2008). The term ''super-shedder'' has been applied to cattle that are high ($> 10^4$ CFU/g feces) shedders of *E. coli* O157:H7 (Matthews *et al*., 2006a, b). Super-shedders could potentially have a substantial impact on the on-farm prevalence and transmission of *E. coli* O157:H7 and the risk of adulteration of food products. Matthews *et al*. (2006b) estimated that super-shedders accounted for 80% of the total *E. coli* O157:H7 shed into the environment by cattle.

The objective of this study was to investigate frequency and duration of super-shedding by cattle. *E. coli* O157:H7 isolated from super-shedding and low-shedding cattle were genetically characterized using pulsed-field gel electrophoresis (PFGE) and lineage typed to evaluate genetic relationships in an attempt to assess the similarity of *E. coli* O157:H7 from super-shedders and low-shedders.

Materials and Methods

All cattle were handled according to the Canadian Council of Animal Care (1993). Crossbred yearling feedlot steers ($N = 400$) from a commercial feedlot in southern Alberta were sampled in July 2011 to identify super-shedders. Cattle from 4 pens were restrained in a chute and a fecal grab sample (50 g) was collected from the rectum. All fecal samples were collected in sterile tubes, placed on ice, and transported to the laboratory for analysis within 4 h. Eleven super-shedders (shedding $\geq 10^4$ CFU/g feces) were identified and transported (35 km) to the Lethbridge Research Centre (LRC) after enumeration was complete (4 days). Super-shedders were then housed individually at the LRC feedlot.

The 34-d study involved a 7-d sampling period where steers were sampled twice daily by collecting freshly voided fecal pats 2 h before and 6 h after feeding, with sampling continuing daily for an additional 19 d. Five of the 11 steers, exhibiting the highest shedding levels, were shipped for slaughter for detailed analysis of intestinal populations and collection of tissues throughout the digestive tract.

E. coli O157:H7 was enumerated and confirmed from fecal subsamples (50 g) according to Hallewell *et al*. (2012). When *E. coli* was not detectable by plating, duplicate 1-g subsamples of feces were enriched and subjected to immunomagnetic separation.

[1]Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, Canada.
[2]Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada.
[3]Alberta Agriculture and Rural Development, Lethbridge, Alberta, Canada.

A total of 126 isolates were recovered over the 34-d period, and lineage was typed using lineage-specific polymorphism assay as outlined by Yang *et al.* (2004). Isolates were PFGE genotyped using a standard protocol (Centers for Disease Control and Prevention, 2000). Isolates that were >90% related were considered highly related.

Results

Of the cattle at the commercial feedlot ($N=400$), a total of 46 (11.5%) were identified as shedding *E. coli* O157:H7, of which 11 (23.9%) of these cattle were super-shedders. Shedding in super-shedders ranged from 1.5×10^4 to 6.5×10^7 CFU/g feces. Once transported to the LRC feedlot, only 5 of the steers were identified as super-shedders over the first 5 days of the study, with no super-shedders being detected 6 d after the first sampling (Table 1). Even within the same steer, super-shedding was not consistent between fecal samples collected in the morning versus the afternoon.

After lineage typing, 99.2% (125/126) of the isolates were lineage I (111111), with only 1 isolate being classified as lineage type 211111. There were three distinct PFGE restriction endonuclease digestion clusters identified, with 97.6% (123/126) of the isolates members of 1 dominant subtype.

Discussion

To date, researchers have sampled cattle biweekly (Jacob *et al*., 2010), weekly (Cristancho *et al*., 2008) and twice weekly (Cobbold *et al*., 2007) to assess the persistence of *E. coli* O157:H7. More intensive sampling has been carried out by Robinson *et al*. (2009) whereby levels of *E. coli* O157:H7 in feces of weaned calves ($N=14$) were sampled every 3 h over 5 days. In that study, it was found that variation within-animal was greater than between animals over time. The pattern or duration of shedding in super-shedders has not been characterized by repeated sampling from the same animal on several consecutive days. In our study, super-shedding was a short-lived isolated event and was not consistent even within the same animal over the course of a day. A number of factors other than the intermittent nature of shedding of *E. coli* O157:H7 by feedlot cattle could contribute to this variability including the feedlot selected, the sampling season, or even year-to-year differences in pathogen prevalence.

While all three lineages of *E. coli* O157:H7 have been found in cattle, only lineages I and the intermediate lineage are typically associated with human disease (Sharma *et al.,* 2009). In this study, all isolates were lineage I (99.2%) or lineage I/II (0.8%), suggesting that cattle were shedding *E. coli* O157:H7 that could cause human illness. Subtyping using PFGE demonstrated that there was a high degree of relatedness among isolates collected on a single day from super-shedder cattle in separate pens within a single feedlot. Previous research that collected fecal samples from multiple feedlots over a period of months showed greater diversity among isolates recovered from super-shedders (Stanford *et al*., 2012).

Conclusion

In conclusion, this study suggests that super-shedding may not play as great a role in transmission and contamination

TABLE 1. *ESCHERICHIA COLI* O157:H7 DETECTION (LOG_{10} COLONY-FORMING UNITS [CFU]/G FECES) FROM CATTLE THROUGHOUT 34-DAY TRIAL

Day																																	
		4		*5*		*6*		*7*		*8*		*9*		*10*		*11*																		
Steer	*0*	*AM*	*PM*	*AM*	*PM*	*AM*	*PM*	*AM*	*PM*	*AM*	*PM*	*AM*	*PM*	*AM*	*PM*	*AM*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*29*	*30*	*31*	*32*	*33*	*34*
219	**5.1**	+	**—**	3.2	–	–	–	–	–	–	–	–	–	2.7	–	–	–	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–
222	**4.2**	–	–	–	3.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
236	**6.0**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
261	**5.1**	–	3.2	–	3.0	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
274	**6.7**	3.5	**8.0**	3.5	3.0	–	+	3.2	3.0	3.5	+	Slaughtered																						
287	**5.4**	2.7	2.7	3.2	2.7	+	–	+	–	+	+	Slaughtered																						
294	**5.8**	–	–	2.7	**4.5**	–	–	–	–	–	–	–	–	3.4	3.0	Slaughtered																		
299	**7.8**	–	**4.4**	–	–	–	–	+	+	–	–	–	–	+	–	Slaughtered																		
310	**7.5**	**5.8**	Slaughtered																															
342	**5.2**	–	–	3.3	–	–	–	–	–	–	–	+	–	+	3.5	3.7	–	+	+	–	–	–	–	–	+	–	–	–	+	–	–	–	–
651	**6.1**	**4.1**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–

Values ≥ 4.0 log_{10} CFU/g feces were considered super-shedders.
AM, 2 h before feeding; PM, 6 h after feeding; +, positive for *E. coli* O157:H7 via immunomagnetic separation; –, no *E. coli* O157:H7 detected; slaughtered, animal was killed as part of a larger trial; bold text identifies super-shedding levels.

within the feedlot environment as has been previously proposed, as the super-shedding state is short lived and lacks continuity. Furthermore, there was a high degree of relatedness among *E. coli* O157:H7 isolated from super-shedder cattle within a single feedlot, and this genotype persisted as cattle transitioned from super- to low-shedders. This may imply that super-shedding is more a function of the time a sample is collected than it is to the nature of the *E. coli* O157:H7 subtype collected or the characteristics of the host.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Tim A. McAllister, PhD
Agriculture and Agri-Food Canada
Lethbridge Research Centre
5403 1st Avenue South
Lethbridge, Alberta, T1J 4B1
Canada

E-mail: tim.mcallister@agr.gc.ca