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Isolation of *Listeria* spp. from Feces of Feedlot Cattle

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ABSTRACT

Healthy feedlot beef cattle were surveyed for the presence of *Listeria* spp. in fecal grab samples taken over 3 months. Composite samples were made from 224 individual animals each month. *Listeria monocytogenes* was isolated from one composite sample (4%) from the first sampling and not from the subsequent two. *Listeria innocua* was found in composite samples from all three samplings at levels of 17, 9, and 35%, respectively. From the individual samples comprising the *Listeria* spp.--positive composites, *L. monoytogenes* was isolated from one sample (3%) in the second sampling but not in the first or third samplings. *L. innocua* was found in 9, 8, and 10% of the individual samples comprising *Listeria*--positive composites in the first, second, and third samplings, respectively. The two *L. monocytogenes* isolates were pathogenic to mice. Further characterization of these isolates revealed atypical rhamnose fermentation patterns. These results indicate that the frequency of isolation of *L. monocytogenes* from feedlot beef cattle is low.

The bacterium *Listeria* is widespread in nature. These organisms are frequently isolated from a large variety of environmental, food, plant, and animal sources (*4,7,12*). Because recent outbreaks of foodborne listeriosis have been linked to dairy and meat products, attention has been directed to identifying animal reservoirs (Table 1) of listeriae in order to better understand the transmission of the disease (*7*). Several workers have screened dairy and beef cattle for fecal carriage of the organism (*2,9,14,15,17,26*). *Listeria* screening has been reported for swine (*2,26,27*), sheep (*13,20,21*), poultry and goats (*2,5,9,18*).

Fecal material is considered to be a major source of pathogen contamination of the final product as well as the processing environment (*1*). Because data are available on the incidence of *L. monocytogenes* and *Listeria* spp. in dairy cows and other red meat animals, this study was done to screen, specifically, feedlot cattle for fecal carriage of *L. monocytogenes* and *Listeria* spp.

[1]*Mention of a trade name, proprietary product, or specific equipment is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.*

MATERIALS AND METHODS

Test animals

A total of 224 cattle (Hereford and Angus crossbred steers) were penned in 22 groups of 10 and one group of four in the U.S. Meat Animal Research Center's (MARC) outdoor feedlot complex located in Clay Center, NE. All test animals were clinically healthy and were 9-12 months old at the start of the study which began in March and was carried on for 3 months. Animals were fed a diet of corn-silage (25%), corn (70%), and protein-mineral concentrate (5%). The feed concentrate was supplemented with the ionophore antibiotic monensin.

Experimental design and sampling plan

The animals were sampled once a month for 3 months beginning in March. At the time of each sampling, a fecal grab sample was taken from each animal as it was caught in a chute. Disposable plastic gloves were changed between each animal. The samples were stored in sterile Whirl-Pak bags and held frozen at -20°C until analyzed.

Samples were thawed and 10 g of feces from each animal within the pen was combined into a 100-g composite sample in a sterile plastic wide-mouth bottle. Nine hundred milliliters of University of Vermont medium (UVM)-2 broth (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, MD) were poured over the sample, mixed by vigorous shaking, then incubated for 24 h at 35°C.

Listeria assays

A sample was considered positive if a *Listeria* spp. was isolated from either the composite enrichment or the individual enrichment (i.e., culture confirmed).

Samples were screened for the presence of *Listeria* spp. using the Gene-Trak *Listeria* gene-probe and assay system (Gene-Trak Systems, Framingham, MA). Since this system was not designed for fecal analysis, the initial enrichment was modified by enriching the samples directly in UVM-2 broth as described above. An 0.1-ml aliquot from each enrichment was spread plated onto LiCl-phenylethanol-moxalactam (LPM) agar (BBL Microbiology Systems) and incubated for 48 h at 37°C. Growth from this plate was harvested with 1.0 ml of phosphate-buffered saline (PBS) and processed according to the manufacturer's instruction.

Culture confirmation on the composite samples, which were positive for *Listeria* spp. in the gene-probe assay, was conducted as follows: (i) an aliquot from the PBS suspension of growth from the LPM plates was streaked onto LPM and/or *Listeria* selective isolation agar (Oxford formulation, Oxoid Unipath Co., Ogdensburg, NY) and incubated for 24-48 h at 37°C; (ii) at least

TABLE 1. *Frequency of* Listeria *isolation from domestic animals feces by various workers.*

Species	Status[a]	L spp.[b]	Lm	Other L spp.	Ref.
Cattle	NR	2/24 (8)	0/24 (0)	2/24 (8)	*8*
Cattle	NR	30/52 (58)	10/52 (19)	20/52 (38)	*2*
Cattle	D	51/75 (68)	39/75 (52)	21/75 (28)	*25*
Cattle	NR	32/300 (11)	32/30 (11)	NR	*16*
Cattle	S, D	287/2256 (12)	207/2256 (9)	NR	*14*
	No S, D	86/1622 (5)	51/1622 (3)	NR	*14*
Cattle	Rg	11/61 (18)	11/61 (18)	NR	*13*
Chickens	NR	97/2373 (4.1)	97/2373 (4.1)	NR	
Goats	Listeric[c]	5/14 (36)	5/14 (36)	NR	*17*
	Healthy	6/48 (13)	6/48 (13)	NR	*17*
Sheep	NR	0/3 (0)	0/3 (0)	0/3 (0)	*20*
Sheep	Pre-lamb	2/232 (<1)	2/232 (<1)	NR	*12*
	Lamb	74/106 (64)	74/106 (64)	NR	*12*
Swine	NR	2/30 (7)	0/30 (0)	2/30 (7)	*8*
Swine	NR	48/97 (50)	3/97 (3)	45/97 (46)	*2*
Swine	S	7/172 (4)	3/17 (2)	4/172 (2)	*26*

[a]Reported management/health conditions. D = dairy animals, S = silage fed, Rg = range fed, Lamb = lambing period, NR = information not reported.
[b]Frequency expressed as *Listeria*-positive samples/total samples tested. Percentages are in parentheses. L = *Listeria* spp.; Lm = *L. monocytogenes*; Other L spp. = *L. innocua*, *L. welshimeri*.
[c]Samples from goats in herd with listeriosis. Healthy goats were asymptomatic and from same herd.

five colonies with the typical *Listeria* spp. morphology and characteristics for each selective agar medium were picked, isolated, and saved for identification. For culture confirmation of the second and third sets of samples, only LPM agar was used.

All individual samples from those composites which were gene-probe assay positive were rethawed and enriched by adding 90 ml of UVM-2 broth to a 10-g sample in a Whirl-Pak bag. Following enrichment for 24 h at 35°C, the sample was screened for *Listeria* spp. as described above.

Isolate identification/characterization

All suspect isolates were identified according to the methods of Lovett (*19*) and Siragusa and Nielsen (*25*). A fluorescent antibody test was performed on the *L. monocytogenes* isolates using Difco (Difco Laboratories, Detroit, MI) polyclonal fluorescein labelled anti-*Listeria* serotypes 1 and 4 antibody according to the manufacturer's instructions. The Mini-Tek system (BBL Microbiology Systems) was used to confirm sugar fermentation tests. All isolates identified as a *Listeria* spp. were tested for reactivity with a monoclonal antibody specific for *L. monocytogenes*, *L. innocua*, and *Listeria welshimeri* using an enzyme-linked immunosorbent assay format (*24*). Reagents for serological typing were obtained from Difco Laboratories. Mouse pathogenicity testing was performed according to Lovett (*19*). Male Swiss Webster mice were obtained from Dominion Labs (Omaha, NE).

RESULTS

The frequency of *Listeria* spp. isolated from feedlot cattle feces ranged from 8.7 to 35% (avg. = 22%) of composited samples over a 3-month period (Table 2). *L. monocytogenes* isolation from these same samples ranged from 0 to 4.4% (avg. = 2.2%). *L. monocytogenes* was isolated from two samples on two sampling dates (Table 2). The remainder of isolates were identified as either *L. innocua* or *L. welshimeri*.

Upon observing a positive result from the gene-probe-based assay of the composite enrichment, the individual fecal samples comprising that composite were thawed out and each individual sample enriched. These assays were done to try to identify a consistent *Listeria* spp. shedder in the pen corresponding to the gene-probe-positive composite sample. Results shown in Table 3 demonstrate that not all sample enrichments could be culture confirmed with the methods used in this study. Seven of 16 gene-probe positive samples were confirmed in both the composite and individual enrichments, whereas only five and six composite and individual enrichments, respectively, could not be culture confirmed.

Of the individual fecal samples enriched, *Listeria* spp. could be isolated from between 8 and 10% of the samples (Table 2). As in the composite enrichment cultures, the other species isolated were either *L. innocua* or *L. welshimeri*.

Table 4 lists the species identification of the isolates made. Characteristics for identification were based on those previously reported (*23*). *L. monocytogenes* is typically known to ferment rhamnose; however, the two organisms identified as *L. monocytogenes* isolated in this study were both rhamnose negative when tested in both the standard tube format (*19*) as well as the modified microtiter plate method (*25*) and the Mini-Tek systems. The isolates belonged to serotype 4 and were positive in the *Listeria*

TABLE 2. *Isolation of* Listeria *spp. from composite and individual fecal samples from feedlot cattle from March through May.*

Sample type	Number of *Listeria*-positive samples[a] Sampling No. 1	2	3
Composite			
Listeria spp.	4/23 (17)	2/23 (9)	8/23 (35)
L. monocytogenes	1/23 (4)	0/23 (0)	0/23 (0)
Other *Listeria* spp.[b]	4/23 (17)	2/23 (9)	8/23 (35)
Individual			
Listeria spp.	3/34 (9)	4/40 (10)	6/64 (9)
L. monocytogenes	0/34 (0)	1/40 (3)	0/64 (0)
Other *Listeria* spp.	3/34 (9)	3/40 (8)	6/64 (9)

[a]Samples were considered positive only after culture method confirmation from either a composite or an individual enrichment. Data are presented as the number of *Listeria*-positive samples/number total samples. Percentages are in parentheses.
[b]Other *Listeria* spp. refer to either *L. innocua* or *L. welshimeri*.

TABLE 3. *Qualitative* Listeria *screening results at each sampling as determined by three methods (see Materials and Methods section).*

Composite	Sampling 1	2	3
1	N[a]	N	N
2	N	N	N
3	P-P-N[b]	N	N
4	N	N	P-P-P
5	N	N	N
6	N	N	N
7	N	N	N
8	N	N	N
9	P-P-P	P-N-P	P-P-P
10	N	N	N
11	N	P-N-P	N
12	P-P-P	N	N
13	N	N	P-N-P
14	N	N	N
15	N	N	P-P-P
16	N	N	N
17	N	P-N-N	P-P-P
18	N	N	P-P-P
19	N	N	N
20	N	P-N-N	P-P-N
21	N	N	N
22	N	N	N
23	P-P-N	N	P-P-N

[a]N = negative result in Gene-Trak *Listeria* gene-probe assay. No further testing was performed on gene probe negative samples.
[b]Results were scored in the following order: Gene-Trak Listeria gene probe assay, composite enrichment culture method, individual sample enrichment culture method.
N = negative result in Gene-Trak *Listeria* gene-probe assay.
P = positive for *Listeria* spp. isolation.

fluorescent antibody test. As further proof of identity, these two isolates were found to be pathogenic in the mouse pathogenicity test (*19*), reacted with a monoclonal antibody specific for *L. monocytogenes*, *L. welshimeri* and *L. innocua* (*24*), and fermented alpha-methyl-d-mannopyranoside (*23*). These last three tests distinguished these isolates from *Listeria seeligeri*.

TABLE 4. *Identification of feedlot cattle* Listeria *spp. isolates and their rhamnose reactions.*

Sampling	Individual (I) or composite (C)	Species	No. isolates	Rhamnose rxn.
1	C 3	*L. innocua*	1	(+)
			8	(-)
	C 9	*L. innocua*	5	(+)
			5	(-)
	C12	*L. innocua*	9	(-)
		L. monocytogenes	1	(-)
	C 23	*L. innocua*	10	(-)
	I 84	*L. innocua*	1	(+)
		L. innocua	3	(-)
	I 114	*L. innocua*	1	(-)
	I 116	*L. innocua*	1	(-)
2	I 81	*L. monocytogenes*	2	(-)
	I 101	*L. innocua*	1	(-)
	I 102	*L. innocua*	2	(-)
	I 105	*L. welshimeri*	3	(+)
3	C 4	*L. innocua*	1	(+)
	C 9	*L. innocua*	1	(-)
	C 15	*L. innocua*	1	(-)
		L. innocua	1	(+)
	C 17	*L. innocua*	1	(+)
	C 18	*L. innocua*	1	(+)
		L. innocua	1	(-)
	C 20	*L. innocua*	2	(+)
	C 21	*L. innocua*	1	(+)
		L. innocua	1	(-)
	C 23	*L. innocua*	2	(+)
	I 33	*L. innocua*	1	(+)
	I 89	*L. innocua*	1	(+)
	I 128	*L. innocua*	2	(+)
	I 150	*L. innocua*	2	(+)
	I 167	*L. innocua*	1	(+)
	I 178	*L. innocua*	1	(+)
Survey totals		*L. innocua*	Rhamnose	(+) 24
				(-) 44
		L. monocytogenes	Rhamnose	(+) 0
				(-) 3
		L. welshimeri	Rhamnose	(+) 3
				(-) 0

DISCUSSION

The frequency of *L. monocytogenes* found in this population of feedlot cattle is similar to that reported by other workers for cattle, sheep, and swine managed under various other conditions (Table 1). Feeding a high portion of silage or silage of poor quality has been linked to the high incidence of fecal *Listeria* spp. excretion, as well as the disease listeriosis in animals (*8,12,20*). The cattle in this study were fed corn silage as 25% of their standard diet.

A single consistent fecal *Listeria* spp. shedder was not found by methods used in this study. Composites made

from animals in pen 9 were positive in all three samplings, but no individual animal was determined to consistently shed listeriae. Clinically, the health status of all animals remained good throughout the survey.

Isolation of *Listeria* spp. from feces was not consistent (Table 3). This was probably due to several factors which were compounded by an already low incidence of the organism. These factors include the extremely high microbial load of feces. It is estimated that feces can contain between 10^{10}-10^{11} CFU/g of bacteria, and specifically in cattle, between 10^4 to 10^5 *Streptococcus* spp. per g (*3,6*). Another factor is freeze-thaw cycling of the samples during the analysis. It has been reported that up to 80% of the populations of *L. monocytogenes* undergo sublethal cellular injury upon freezing at -18°C (*10*). Additionally, bacteria which were inhibitory to *Listeria* spp. were evident by frequently observed zones of inhibition surrounding colonies amongst the background *Listeria* growth on the LPM *Listeria* selective agar plates (Fig. 1, photo). Several of these bacteria were isolated and found to be Group D enterococci ("fecal streptococci"). Enterococci are not inhibited by the selective broth used (UVM-2) as evident by their high numbers when the UVM-2 enrichments were plated on KF-Streptococcal agar (data not shown). Only two gene-probe-positive samples were not culturable either from the composite suspension or the individual enrichment (Table 3). A two-stage enrichment procedure may have eliminated this inconsistency.

The inability of the two *L. monocytogenes* isolates to ferment rhamnose is unusual considering data reported elsewhere (*22,23*). In their survey of animals and meat samples, Skovgaard and Morgen (*26*) reported *Listeria* spp. were isolated from poultry samples with atypical carbohydrate patterns, but the specific irregular reactions were not stated. In the case of *L. innocua* and *L. welshimeri* isolates made from enrichments in this study, rhamnose fermentation was variable (*23*). The significance of this finding is not clear at this point but is noteworthy since the two *L. monocytogenes* isolates were found to be pathogenic to mice and were from two different composite samples.

The results of this survey give further evidence that the incidence of *Listeria* spp. in meats is probably due more to the common presence of the organism in processing environments, rather than a high incidence of the organism in live animals (*2,11,16,21*).

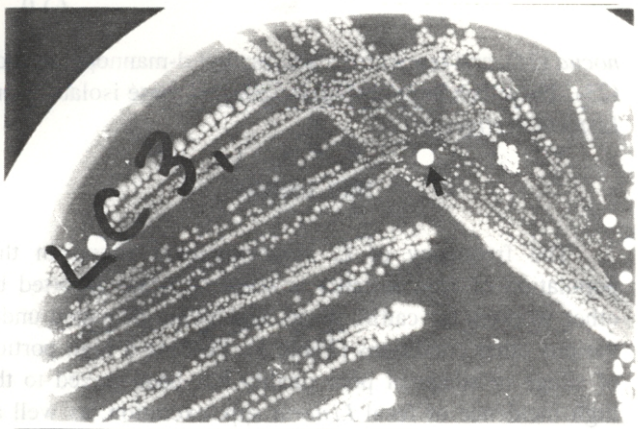

Figure 1. *Photograph of LPM agar streak plate of UVM-2 enrichment of composited sample 3 from the first fecal sampling date. Note clear zone of inhibition surrounding inhibitory colony. Background growth was found to contain* L. innocua *(see Table 3).*

It is interesting that the species of listeriae found most often in this study were isolates of *L. innocua*. This finding is consistent with most other studies on the incidence of *Listeria* spp. in meat animals (see Table 1) and red meats (*16*). The significance of this relationship is not known at this time.

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