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A case report of sporadic ovine listerial menigoencephalitis in Iowa with an overview of livestock and human cases

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Abstract. A case of ovine listeriosis was examined in a flock of sheep. The index case was a male lamb, which was part of a flock of 85 sheep located in central Iowa. Because the sheep were raised on a premise where soybean sprouts were also cultivated for the organic foods market, the potential of a public health concern was addressed. To identify the source of contaminations, clinical and environmental samples were cultured for *Listeria monocytogenes.* Isolates were serotyped and analyzed using pulsed-field gel electrophoresis (PFGE). *Listeria monocytogenes* (serotype 1) was recovered from the brain of a male lamb with clinical signs of listerial encephalitis. Isolates of serotypes 1 and 4 were also cultured from feces of clinically healthy lambs, compost piles, and soybean cleanings. By PFGE, the clinical isolate was distinctly different from the other isolates. Environmental isolates were identified as *L. monocytogenes* serotypes 1 and 4. However, by PFGE, none matched the profile of the single clinical isolate. Thus, the ultimate source of contamination is unknown.

Listeria monocytogenes is a Gram-positive, nonsporulating rod, which produces encephalitis, septicemia, and abortion in both humans and livestock. *Listeria monocytogenes* is a major human bacterial foodborne pathogen that annually accounts for an estimated 2,500 cases (meningitis, encephalitis, sepsis, fetal death, premature births) and 504 deaths in the USA at an estimated loss of $200 million.[8] Nearly 90% of all reported cases result in hospitalization. The mortality rate of clinical listeriosis (~25%) is the highest of all food-borne illnesses.[24] The US Public Health Service has set the goal of reducing human bacterial foodborne illness. Listeriosis cases in the USA are projected to decline by ~50% from the 1997 baseline (0.5 cases per 100,000 population) by year 2010 (0.25 cases per 100,000 population).

Livestock are also susceptible to listeric infection, with a large proportion of healthy asymptomatic animals shedding *L. monocytogenes* in their feces.[16,29,36,39] Sheep, cattle, goats, and, less frequently, poultry are susceptible.[28,41] While the majority of infections are subclinical, listeriosis in animals can occur either sporadically or as epidemics. Listeriosis is reported most frequently in temperate and cold climates during the fall, winter, and early spring.[19] Although it is not a notifiable disease, reports of listeriosis in domestic animals have increased worldwide, including in England, New Zealand, Germany, and Eastern Europe, which may reflect fluctuations in the distribution of susceptible hosts, changes in silage production, and/or improved detection methods.[1] In Canada, the majority of listeriosis cases occur in bovine (82%), with a smaller percentage from sheep (17%) and fewer still from pigs.[3] From 1975 to 1984, listeriosis cases in Great Britain were primarily from sheep (63%) and cattle (32%), with pigs, goats, fowl, and other species constituting less than 1% each of the total submissions.[48] This may reflect differences in relative proportions of livestock populations.

The excretion of *L. monocytogenes* by sheep has been linked to diet, especially consumption of poorly prepared silage.[4,16,38] In Great Britain, a significant association between silage feeding and an increased relative risk (3.8) for development of ovine listeric encephalitis was determined.[48] The incidence of *L. monocytogenes* in sheep and cattle declined in The Netherlands due in part to the change of silage production; in contrast, in Great Britain, alterations in silage production may have preceded an increase in ovine listeriosis.[48]

Ovine listeriosis is usually classified as 1) encephalitis, which is the most common form, 2) placentitis with abortions occurring in the last trimester, and 3) septicemia.[28,41] In the UK, meningoencephalitis caused by *L. monocytogenes* is the most common bacterial infection of the central nervous system of adult sheep.[35] Lambs as young as 5 weeks may develop the

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septicemic form, with older feedlot lambs (4–8 months) developing the encephalitic form. Ovine outbreaks (>5 cases) are predominantly of serotype 4b[18]; sporadic isolated cases are mainly attributed to serotype 1/2.[22,29]

Genetic typing has been used to characterize and demonstrate the possible relationship among isolates of the same serovar recovered from a single outbreak.[9,13,15,40] Genetic analysis has included phage typing,[2] ribotyping,[44,46] pulsed-field gel electrophoresis,[6] or polymerase chain reaction (PCR)-based assays, such as randomly amplified polymorphic DNA (RAPD) as well as amplification of enterobacterial repetitive intergenic consensus (ERIC).[20,23,34,37] Randomly amplified polymorphic DNA analysis was used in an outbreak of ovine meningitis that developed in sheep fed poor-quality silage heavily contaminated with *L. monocytogenes* (10^6 CFU/g). The serovar and phagovar of the *L. monocytogenes* strains isolated from 2 silage samples and the brains of 3 of the 53 affected sheep were indistinguishable, inferring a causal association.[38] In another report, strains from both the silage and the livestock (1 each of sheep, cattle, and goat) recovered from 3 of the 4 outbreaks exhibited the same ribotype, which pointed to a causal association between consumption of the silage and clinical disease.[46] Identity of genetic profiles obtained by RAPD analysis linked *L. monocytogenes* 1/2a present in the silage to an outbreak in sheep. In contrast, by RAPD analysis, isolates from a goat brain and silage differed, which suggested that the source of *L. monocytogenes* was not silage.[47]

In humans, *L. monocytogenes* has been implicated in major food-borne epidemics. For some of these outbreaks, indirect transmission of the pathogen from animal to the food product has been suggested. In an outbreak attributed to coleslaw, it was postulated that manure collected from sheep with clinical signs of listeriosis was used to fertilize cabbage, which was later used to make coleslaw.[33] In a New England outbreak involving several foods, including dairy products, it was suggested that cows that supplied the suspect milk were from a herd where clinical signs of suggestive listeriosis had been noted.[12] In the California Mexican-style soft-cheese epidemic, pasteurized milk was mixed with raw milk obtained from cows that were presumed to be clinically healthy carriers.[21] For none of these episodes was an isolate from suspect livestock ever obtained to support the hypothesis.

The current study was prompted by a case of fatal ovine listeriosis, which occurred on a premise in the Midwest that was also growing soybean sprouts for the organic health food market. It has been documented that contaminated alfalfa sprouts have caused human outbreaks of *Escherichia coli* O157:H7[7] and *Salmonella*.[27] The recovery of *L. monocytogenes* from a sheep brain following an earlier death of a lamb and the report of several feeder lambs on the premises with symptoms compatible with listeriosis raised public health concerns. In order to find a possible relationship between animal and human listeriosis, we reviewed submissions to the Iowa State University Veterinary Diagnostic Laboratory and the Iowa Department of Public Health from 1993 through 2000.

Materials and methods

Clinical findings. In November 1997, a 6-mo-old male lamb (100 lbs.) from a herd of 85 sheep in southwest Iowa exhibited mild anorexia. Twenty-four hours later, the animal was found in lateral recumbency with total facial paralysis, at which time the animal was euthanized. The lamb had been vaccinated with 2 doses of *Clostridium* C and D toxoid and tetanus toxoid at 3–4 mo of age. At necropsy, no significant gross lesions were seen in the brain, thoracic organs, or abdominal organs. One week earlier, another lamb had died with similar clinical signs; several other lambs were mildly anorexic. The lambs were fed soybean hulls and other soybean products.

Clinical submissions. Ovine tissues (brain, ileum, duodenum, liver, kidney), whole blood, and serum were obtained from the lamb, which was designated case 37435. Histopathology, immunohistochemistry, and bacteriology were performed on the brain. The sheep brain was fixed in buffered 10% formalin, dehydrated, and embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin (HE). For immunohistochemistry, 5-μm sections were labeled with polyclonal antibodies for *Listeria*[a].

Bacterial isolation. For isolation of *Listeria*, brain tissues were plated to tryptose blood agar base supplemented with 5% defibrinated bovine blood and incubated in aerobic, anaerobic, and microaerobic (6% CO_2) environments (up to 48 hr, 37 C). Presumptive *Listeria* colonies (small and beta hemolytic) were Gram stained, biochemically phenotyped, transferred to blood agar slants (24 hr, 37 C), and refrigerated. For cold enrichment, brain tissues were passed through a 3-ml syringe into University of Vermont (UVM)-modified *Listeria* enrichment broth[b] (10% w/v). Every week for 12 wk, an aliquot was plated to *Listeria*-selective agar and incubated (24 hr, 37 C). Presumptive *Listeria* colonies, which appeared as black colonies against a clear yellow/green background, were restreaked onto blood agar and incubated (24 hr, 37 C). Suspect colonies were Gram stained, biochemically phenotyped, transferred to blood agar slants, incubated (24 hr, 37 C), and refrigerated.

Toxicology was performed for whole-blood lead and aqueous fluid nitrate. Sections of the intestinal tract were cultured for *Salmonella, E. coli,* and *Pasteurella*.

Farm management. The water supply consisted of pond and well water, which was known to be high in nitrates and coliforms. Lambs were given standard sheep rations and soybean hulls and had free access to soybean cleanings as well as compost piles on the premises (Fig. 1).

Environmental isolates. Samples were collected from the premise within 1 mo after the initial diagnosis of listeriosis

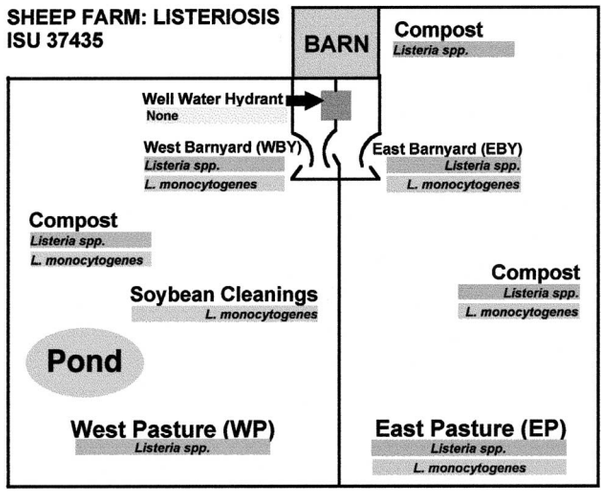

Figure 1. Schematic of premises from which listeriosis was reported in sheep. Placement of water, compost piles, and soybean cleanings is indicated.

in the feeder lambs. Samples of feces (n = 24), water (n = 2), feed (n = 1), soybean compost (n = 4), and fermented soybean cleanings (n = 1) from the herd were cultured for *L. monocytogenes.* The samples were shipped overnight on ice to the National Animal Disease Center, Ames, Iowa. Samples (1 g) were suspended in UVM I (9 ml) and incubated (48 hr, 32 C). An aliquot (100 μl) was then transferred to UVM II (9.9 ml). Following incubation (48 hr, 32 C), a loop of UVM II was plated to Palcam agar (24 hr, 32 C). The presence of colonies surrounded by a black halo was used for initial identification of *Listeria.* Up to 5 presumptive *Listeria* colonies from each plate were picked, subcultured to trypticase soy agar supplemented with 0.6% yeast extract (TSA-YE), and refrigerated for subsequent confirmation by Gram stain, PCR, serotyping, and genetic analysis.

PCR amplification. Two primer sets were used: set I (primers Lis −1 and Lis 2) and set II (primers U1 and LI 1). Primers Lis-1 (5′-GCA-TCT-GCA-TTC-AAT-AAA-GA-3′) and Lis-2 (5′-TGT-CAC-TGC-ATC-TCC-GTG-GT-3′) specifically amplify a 174-bp region of the listeriolysin gene in *L. monocytogenes.*[10] Primers U1 (5′-CAG-GMG-CCG-CGG-TAA-TWC-3′), where M denotes A or C and W denotes A or T, and LI1 (5′-CTC-CAT-AAA-GGT-GAC-CCT-3′) target a 938-bp rRNA sequence in members of the genus *Listeria.*[5] Primers were commercially synthesized.[c]

Amplification was performed in a 50-μl volume containing 0.5 ng sample DNA; 50 pmole each of Lis-1, Lis-2, UI, and LI1; 1.25 U of Taq DNA polymerase[d]; 200 μM (each) dATP, dCTP, dTTP, and dGTP; 10 mM Tris-HCl; 50 mM KCl; and 6.5 mM $MgCl_2$. The reaction mixture was overlaid with mineral oil, and the tubes were placed in a DNA thermal cycler.[e] The samples were subjected to an initial denaturation (94 C for 4 min) followed by 25 amplification cycles. Each cycle consisted of denaturation (94 C for 1 min), primer annealing (60 C for 1 min), and primer extension (72 C for 1 min). A final primer extension step (72 C for 5 min) followed the final amplification cycle. The PCR reaction products were size separated electrophoretically on 1.5% agarose gels (60 V, 1.5 hr) in a horizontal gel bed (8.3 × 6.0 cm) with Tris-borate EDTA (TBE) as the running buffer. The DNA molecular weight marker VI[d] was included for base-pair size comparison. The gel was then stained with ethidium bromide and visualized according to Sambrook et al.[32]

For colony PCR, the above procedure was followed with the following modifications. A purified colony or swipe of culture from a plate was resuspended in 150 μl of sterile distilled water in a microcentrifuge tube. The sample was boiled (110 C for 10–15 min), pelleted[f] (30 sec), and immediately placed on ice. An aliquot of the supernate (5–10 μl) was used as the DNA template and amplified as described above for purified DNA.

Serotyping. The clinical and field isolates of *L. monocytogenes* confirmed by PCR (n = 12) were grouped using a rapid slide test for serotypes 1 and 4,[g] as recommended by the manufacturer.

PCR-based ERIC analysis. ERIC profiles of the field strains were compared with those generated from the clinical ovine strain (NADC 6855). *Listeria monocytogenes* strains were grown on TSA-YE plates and DNA was extracted using High Pure PCR Template Preparation Kit,[d] according to the manufacturer's instructions. Genomic DNA was quantified spectrophotometrically at 260 and 280 nm.[h] All reaction volumes were 50 μl, which contained 0.2 mM of each of 4 dNTPs, 1.25 U of *Taq* DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 25 pmoles each primer, and 50 ng of template (genomic) DNA. Reactions were overlaid with mineral oil. Primers for the ERIC-PCR (ERIC1R, 5′-ATGTAAGCTCCTGGGGATTCAC-3′, and ERIC2, 5′-AAGTAAGTGACTGGGGTGAGCG-3′) were used as described.[27] Amplification conditions included initial denaturation (1 cycle at 94 C, 5 min), followed by 40 cycles of denaturation (1 min at 94 C), annealing (1 min at 25 C), primer extension (4 min at 74 C), and a final extension (74 C for 10 min). Amplification was performed in a DNA thermal cycler.[e] The PCR products were electrophoretically separated (120 V for 45 min) using 1.5% agarose gel and Tris buffered EDTA (TBE) as the running buffer. The gels were stained with ethidium bromide, rinsed, and photographed on a Gel Doc.[i] Resultant patterns were compared visually.

Pulsed-field gel electrophoresis. Environmental strains (n = 12) and the clinical isolate (NADC 6855) were compared using the pulsed-field gel electrophoresis (PFGE) technique of contour clamped homogeneous electric fields (CHEF) electrophoresis as described.[6] Genomic DNA in agarose plugs was separately digested with *Asc*I,[j] as described by the manufacturer. The resulting restriction fragments were separated through Ultrapure agarose[k] (1%) by CHEF-PFGE using a CHEF-DRII apparatus[i] at 200 V and 14 C for 24 hr with switch times ranging from 5 to 40 sec. *Xba*I[j] digests of *E. coli* O157:H7 strain G-5244 were used as a molecular weight standard. In addition to visual inspection, PFGE patterns were analyzed and scored using the Molecular Analyst software[i] as identical (>90% homology), similar (89.5–80% homology), or different (<80% homology).

Results

Histopathology. Examination of the pons and medulla revealed severe suppurative encephalitis charac-

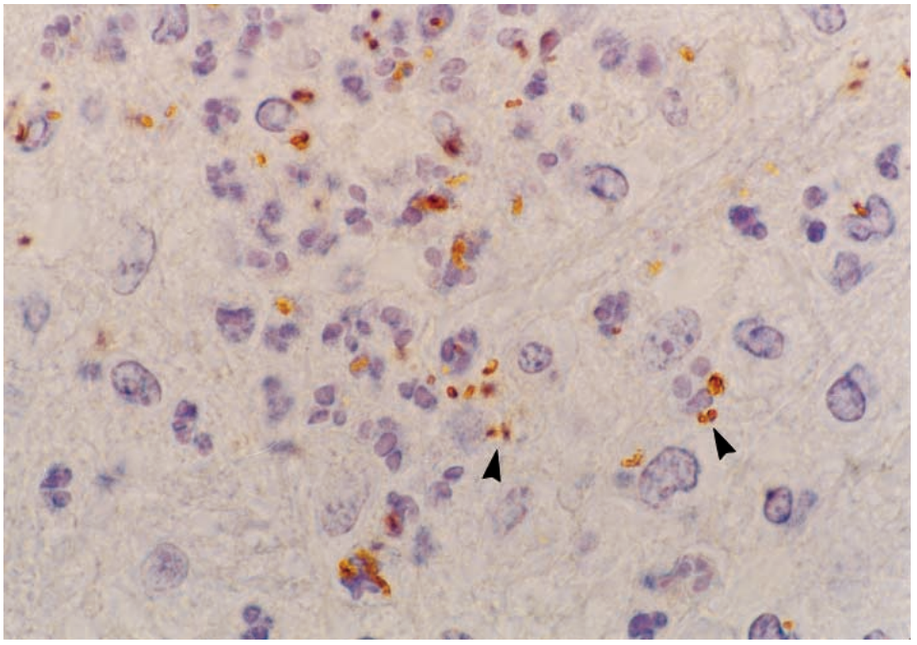

Figure 2. Immunohistochemistry labeling of *Listeria monocytogenes* (arrows) in a microabscess in the brain stem of an ovine.

terized by multiple microabscesses and foci of malacia and axonal degeneration. Multiple perivascular cuffs of mostly mononuclear and some polymorphonuclear inflammatory cells were observed. Special staining revealed low numbers of small Gram-positive bacterial rods within microabscesses compatiable with the diagnosis of encephalitic listeriosis (Fig. 2). No significant lesions were seen in the liver and kidney.

Bacteriology and toxicology. *Listeria monocytogenes* was only recovered from the brain stem and was designated as isolate NADC 6855. Whole-blood lead (<50 ppb) and aqueous fluid nitrate (<50 ppm) levels were within normal range.

Environmental isolates. *Listeria monocytogenes* was recovered from 25% of fecal samples (n = 24), 1 from each compost sample collected from the west and east pastures and from the soybean cleanings. Other *Listeria* species were cultured from 11 of 24 fecal samples and the single feed sample. No isolations of *L. monocytogenes* or other *Listeria* species were made from the well water. Isolates available for further analysis were from soybean cleanings (30b, c, d, e), feces of clinically healthy lambs (4d, 6c, II6c 7b, 13e, 22b, 22c), and from compost on the west pasture (25d).

Serotyping. Environmental strains of *L. monocytogenes* were grouped into serotype 1 (n = 2 strains) and serotype 4 (n = 10 strains). Serotype 1 isolates were recovered from feces of a healthy lamb (22b) and soybean cleanings (30d). The clinical isolate NADC 6855 was assigned to serotype 1.

ERIC. By visual comparison, NADC 6855, the clinical isolate recovered from the case of ovine listeriosis, was distinctly different from the environmental strains (Fig. 3).

PFGE. As shown by the homology matrix, clinical isolate NADC 6855 differed from the environmental strains. It was most closely related (74%) to an isolate (30d) recovered from soybean cleanings and a fecal isolate from a clinically healthy lamb (22b). Both of these environmental isolates were of serotype 1. Clinical isolate NADC 6855 was distinct (<50% homology) from other environmental strains. Visual comparisons of the resulting genomic fingerprints revealed that the 12 environmental strains were comprised of 8 distinct restriction endonuclease digestion profiles (Fig. 4). Groups A, B, and C, which included the clinical isolate 6855, were distantly related to the other environmental strains. Group D (strains 30b from soybeans and 22c from a lamb), group E (strains II6C from a lamb and 30e from composting soybeans), and group G (strains 30c from fermenting soybean cleaning and 13e from a lamb) each contained 2 strains with highly similar profiles. Although the remaining strains displayed distinct profiles, there was appreciable relatedness among the strains. For example, group D was 86% similar to group E; group G was 95% similar to group H (strain 25d from compost).

Listeriosis submissions (1993–2000). This ovine case (case 37435) prompted a review of previous submissions. A summary of listeriosis cases (n = 346) submitted to the Iowa State University Veterinary Diagnostic Laboratory from 1993 to 2000 is shown in

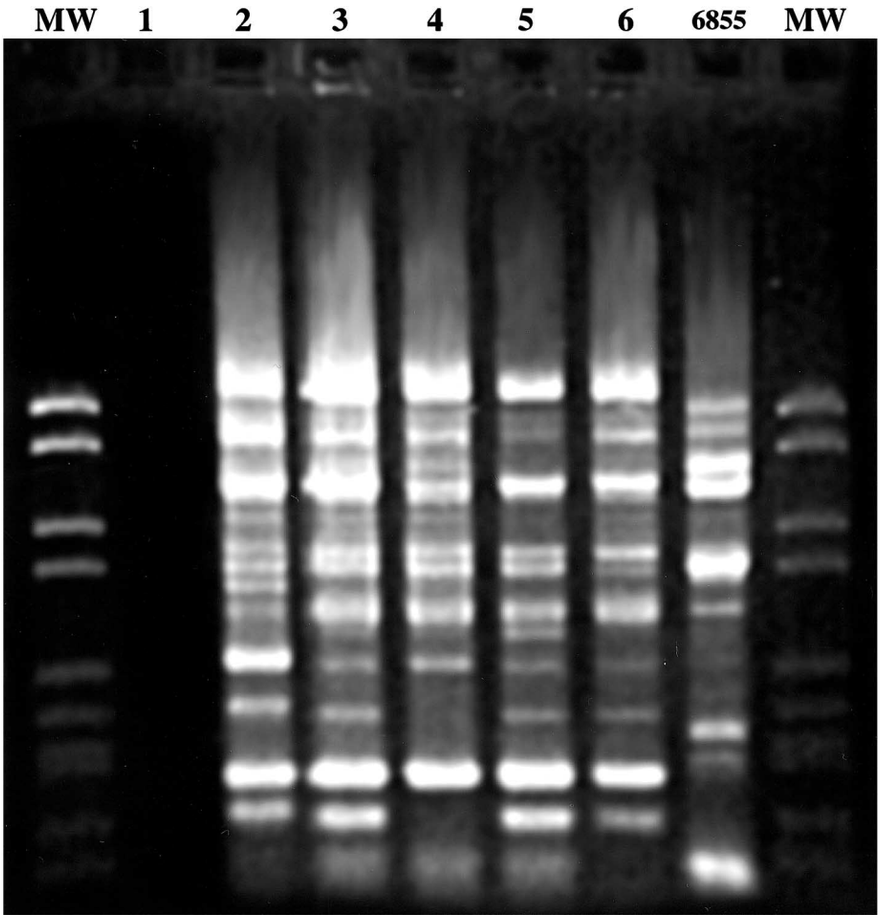

Figure 3. ERIC profiles of field strains (lanes 2–6) differed from that of the clinical isolate NADC 6855. Negative control (lane 1). Compost field isolates 25d (lane 2) and soybean cleanings isolates 30b (lane 3), 30c (lane 4), 30d (lane 5) and 30e (lane 6).

Fig. 5. From 1993 through 2000, 88% of the listeriosis cases (305 of 346) were diagnosed as encephalitis. The majority of the cases occurred in the winter and early spring (December–May). Fewer were reported in the summer and fall months (June–November). During this 8-year interval, clinical submissions were from bovine (85.8%), ovine (9.5%), caprine (3.8%), llama (0.3%), and equine (0.6%), as shown in Table 1. We

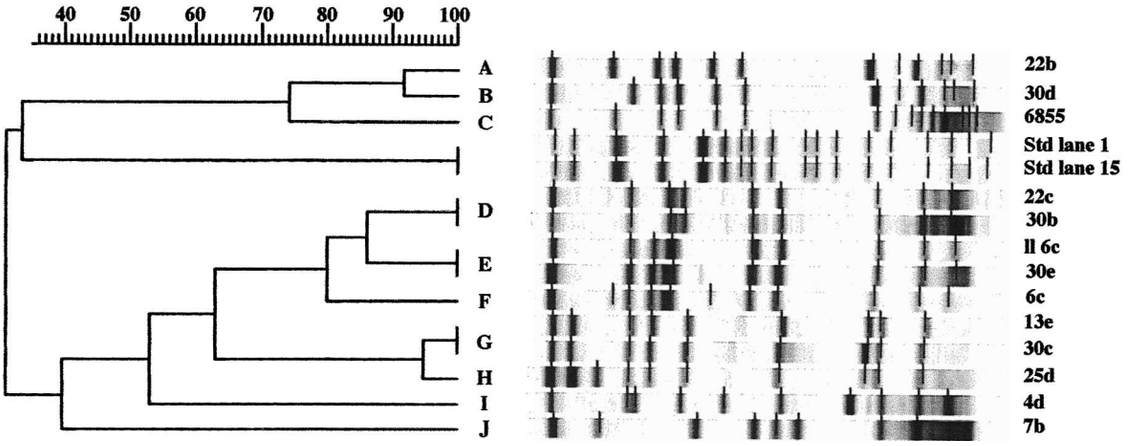

Figure 4. Dendrogram indicating clusters of isolates based on PFGE profiles. Percent homology is indicated on scale.

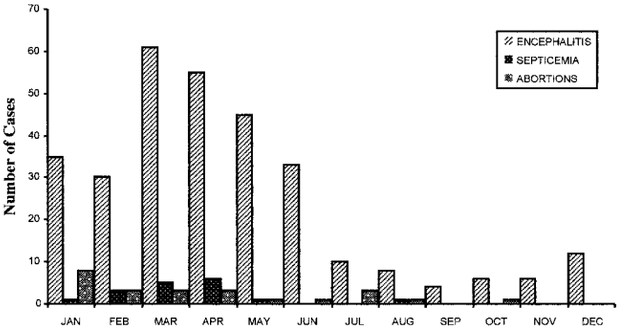

Figure 5. Composite histogram showing the distribution of listeriosis cases submitted to Iowa State University, 1993–2000.

reviewed sporadic human listeriosis cases (n = 12) submitted to the Iowa Department of Public Health from 1990 to 2000. Because none of these human cases clustered as an epidemic (defined as more than 5 cases reported from a single county), no information, including date of occurrence, is available. The overall human mortality rate was 16% due to a total of 2 deaths for the 12 cases.

Discussion

The diagnosis of listeric encephalitis in the male lamb was based on histopathology, including the presence of Gram-positive microbes in brain microabsccesses, the *Listeria*-positive immunohistochemistry test, and the recovery of *L. monocytogenes* from the brain stem. This case was of special interest because the sheep were raised on a premise on which the owner cultivated soybeans for the organic health food market. The lambs were fed soybean hulls. Within 1 month of the ovine listeriosis fatality and receiving laboratory confirmation of listeric meningitis and shortly after field samples were collected, the owner depopulated the herd and vacated the premises, which precluded any other environmental or herd sampling.

The ERIC and PFGE profiles of the clinical isolate NADC 6855 differed from those of the field strains collected on the premises. By PFGE, the clinical isolate was distantly related (74.1% homology) to an isolate from soybean cleanings. Multiple isolates of *L. monocytogenes*, perhaps of differing virulence, may have existed on the premises. The index case was of serotype 1. Isolates (n = 10), which were recovered within a month after the occurrence, from feces, and soybean cleanings were of serotype 4. *Listeria monocytogenes* serotype 4 was recovered from 4 different fecal samples (n = 24), from the compost sample collected from the west pasture, and from the soybean cleanings. The recovery of *L. monocytogenes* serotype 4 from the soybean hulls fed to the sheep suggests that this may have been a risk factor for infection. Interestingly, 3 of the soybean isolates (30b, c, and e) were similar to fecal isolates from clinically healthy lambs, including II6C (88.9% homologous), 25D (94.7% homologous), and 13e (86.67% homologous). This is compatible with previous reports that, even when all sheep are exposed to the same contaminated feed, no more than 5–10% of animals develop clinical listeriosis.[19] Low pathogenicity has also been demonstrated for experimental injections of pregnant ewes with *L. monocytogenes*, after which 10% of inoculated animals aborted, although all developed antibody titers to *L. monocytogenes* and exhibited elevated temperatures and anorexia.[14] In the current study, although 1 confirmed listeriosis fatality occurred, it is highly likely that more sheep were infected, albeit with various strains of *L. monocytogenes.* At the time of this fatality, the owner reported to the attending veterinarian that several lambs exhibited anorexia and 1 lamb died 1 week earlier with clinical signs suggestive of listeriosis.

In the current study, sheep had access to an enclosed area as well as open pasture, compost piles, and soybeans. An interview with the owner did not reveal any single precipitating factor leading to the fatalities. The outbreak occurred during the month of November, which represents a relatively high-risk month for animal infections in Iowa. Ovine listeriosis occurs most frequently in late autumn, winter, and early spring, when animals are maintained in crowded pens.[16,29] Factors such as genetic predisposition, sudden changes in feed, introduction of new animals into the flock, overcrowding, transportation, concurrent disease, climatic changes such as heavy rains, experimental dexamethasone treatment to simulate stress, and physical or viral damage

Table 1. Host distribution of listeriosis cases submitted to the Iowa State University Veterinary Diagnostic Laboratory (1993–2000).

	1993	1994	1995	1996	1997	1998	1999	2000	Total
Bovine	48 (84%)	52 (84%)	43 (86%)	38 (97%)	40 (89%)	30 (70%)	24 (86%)	22 (100%)	297 (85.8%)
Ovine	9 (16%)	7 (11%)	4 (8%)	1 (3%)	3 (7%)	7 (16%)	2 (7%)	0 (0%)	33 (9.5%)
Caprine	0 (0%)	3 (5%)	2 (4%)	0 (0%)	1 (2%)	5 (12%)	2 (7%)	0 (0%)	13 (3.8%)
Equine	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)	2 (0.6%)
Llama	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.3%)
Porcine	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0.0%)
Total cases	57 (100%)	62 (100%)	50 (100%)	39 (100%)	45 (100%)	43 (100%)	28 (100%)	22 (100%)	346 (100.0%)

to the buccal cavity or epithelial lining of the digestive tract may alter the course of infection.[11,17–19,26,30,41,42]

No other livestock were raised on this premise. Earlier reports indicated contamination of ewes' milk by *L. monocytogenes* was significantly higher on farms where cows were also reared compared with farms with only ewes. This suggests either environmental contamination on farms due to *L. monocytogenes* in bovine feces or feeding of contaminated ensilage shared by sheep and cattle.[31] Sheep may be more susceptible to clinical listeriosis than cattle. By molecular analysis, Wiedmann et al. demonstrated that traces of contaminated corn silage left on farm equipment used to feed cattle may have precipitated listerial encephalitis in sheep but not in cattle.[44]

In the USA, the estimated prevalence of human listeriosis cases has remained unchanged.[25] Coincidentally, livestock listeriosis has declined in Iowa from 1993 (57 cases) to 2000 (22 cases), based on records of the Iowa State University Veterinary Diagnostic Laboratory. Genetic analysis of virulence genes *hlyA*, *actA*, and *inlA* indicates that *L. monocytogenes* strains may differ in their pathogenicity for humans and livestock.[45] This supports earlier observations that sporadic listeriosis in livestock may exist independently of human cases.[43]

Acknowledgements

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Sources and manufacturers

a. Fisher Scientific, Itasca, IL.
b. Oxoid, Ogdensburg, NY.
c. Integrated DNA Technologies, Inc., Coralville, IA.
d. Boehringer Mannheim Biochemicals, Indianapolis, IN.
e. Applied Biosystems, Foster City, CA.
f. Microcentrifuge Model 235C, Fisher Scientific, Pittsburgh, PA.
g. Difco Laboratories, Detroit, MI.
h. DU-600, Beckman, Fullerton, CA.
i. Bio-Rad Laboratories, Hercules, CA.
j. Promega Corp., Madison, WI.
k. Gibco BRL, Grand Island, NY.

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