Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

The risk of *Listeria monocytogenes* infection in beef cattle operations

H.O. Mohammed[1], E. Atwill[2], L. Dunbar[2], T. Ward[2], P. McDonough[1], R. Gonzalez[1] and K. Stipetic[1]

1 Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
2 Department of Population Medicine, School of Veterinary Medicine, University of California, Davis, CA, USA

Keywords
beef, cattle, *Listeria monocytogenes*, risk factors.

Correspondence
Hussni O. Mohammed, College of Veterinary Medicine and Diagnostic Sciences, Cornell University, S1 070 Schurman Hall, Ithaca, NY 14850, USA. E-mail: hom1@cornell.edu

2008/1697: received 4 October 2008, revised 1 June 2009 and accepted 4 June 2009

doi:10.1111/j.1365-2672.2009.04446.x

Abstract

Aim: To determine the prevalence of *Listeria monocytogenes* and associated risk factors among beef operations (cow-calf and feedlot) in central and southern California.

Methods and Results: A repeated cross-sectional study where faecal and environmental samples were collected from 50 operations three times a year at different seasons was carried out. Samples were tested for presence of *L. monocytogenes* using a combination of enrichment and polymerase chain reaction tests. Data on putative risk factors were also collected. *Listeria monocytogenes* was detected in faecal samples from cows, calves and other animals on calf-cow operations at proportions of 3·1%, 3·75% and 2·5%, respectively. The organism was detected in 5·3% of cut-grass, 5·3% of soil, 14·3% of irrigation ditches, 3·1% of the ponds and 6·5% of water troughs samples. *Listeria monocytogenes* was less common in faecal (0·3%) and soil (0·75%) samples collected from feedlots.

Conclusions: *Listeria monocytogenes* was present at a higher proportion among cow-calf operations than feedlots. There was no significant seasonal variation in the occurrence of this pathogen within the two types of operations.

Significance and Impact of the Study: If risk mitigation strategies were implemented to reduce the public health risk these should focus in cow-calf operations.

Introduction

Several foodborne outbreaks of gastroenteritis, meningoencephalitis and/or abortion in humans around the world have been attributed to the consumption of dairy and beef products that were contaminated with *Listeria monocytogenes* (Gray 1963; Schwartz *et al.* 1988; Troutt and Osburn 1997). In the United States, the annual incidence of listeriosis has been estimated at 2500 cases and the case-fatality at 20% (Norton and Braden 2007). Consumption of a number of animal-derived foods including beef products has been associated with listeriosis (Schwartz *et al.* 1988; Buncic 1991; Troutt and Osburn 1997; Bailey *et al.* 2003; Gray *et al.* 2004; Madden *et al.* 2007). In addition to the severe diseases in humans, *L. monocytogenes* has been associated with major economic losses in animals causing abortion and encephalitis (Stockton *et al.* 1954; Gray *et al.* 1956; Young and Firehammer 1958; Hathaway 1997).

Sources of infection with this foodborne pathogen in beef cattle are not well known. Several studies around the world attributed the infection in cattle to the system of management including feeding practices (Hofer 1983; Hathaway 1997; Bailey *et al.* 2003; Madden *et al.* 2007). In spite of the abundance of data on the risk of *L. monocytogenes* among dairy herds and dairy products around the world, information on the occurrence of the infection among beef cattle in the United States is scarce (Young and Firehammer 1958). This lack of information is likely to hinder the effort of controlling the incidence of the disease in beef cattle and hence reducing the risk of human listeriosis.

Studies on dairy farms have incriminated silage, hay and bedding as possible sources of exposure to the organism (Ueno *et al.* 1996; Hassan *et al.* 2001; Borucki *et al.* 2005; Hutchison *et al.* 2005a). Furthermore, *L. monocytogenes* has been isolated from surface water in livestock ecosystems (Lyautey *et al.* 2007). These potential sources of exposure have not been investigated in beef operations.

The objectives of this study were to estimate the likelihood of occurrence of *L. monocytogenes* among beef operations and identify the potential sources of exposure within these farms. This information is critical to design cost-effective interventions to help control the risks associated with *L. monocytogenes* in foods containing beef products.

Material and methods

Design and study population

We carried out a repeated cross-sectional epidemiologic study to estimate the likelihood of occurrence of *L. monocytogenes* among beef operations and to identify the potential sources of exposure within these farms. Animals and environmental samples were collected from cow-calf and feedlot operations in the target population (beef operations in central and southern California) at three different times of the year (three seasons). The total number of cow-calf and feedlot operations was obtained from the database kept at Tulare station, University of California Davis. Operations enrolled in the study were selected randomly from this database, and the study sample was stratified by type of production, cow-calf and feedlot operations. A total of 25 farms were selected from each stratum.

Samples and sampling design

Cow-calf operations were visited three times, and faecal samples were collected from 20 calves and cows in the first visit at branding. A similar number were collected before weaning during a second visit and from 40 cows at a dry period during the third visit. Animals to be sampled were selected at each visit, and 50 g of faeces were collected in a rigid plastic container. In addition, seven environmental samples were collected from each cow-calf operation: two from running water, one from standing water, one from a water trough, one from range and pasture forages and two from soil. The running water samples consisted of 100 ml, each collected from a stream on the ranch at entry and exit points. The water samples were collected in a 250-ml bottle. A 100 -ml aliquot was later passed through a filter using a syringe. The filter was then transferred aseptically to a sterile tube. One composite sample of biofilm swabs were collected from all water troughs and placed into a sterile vial. In addition, a composite sample of water from stock ponds in the farm was also collected. The feed samples consisted of cut native forages (annual grass) from two areas in the farm: high risk area (area around the watering zone) and low risk area (a random grazing area). A composite sample of *c.* 250 g of rangeland forage/pasture was cut from the two areas and transferred to two sterile plastic bags. Two types of soil samples were then collected from the ranch. The first was a composite sample soil of scratched surface (1 cm deep) around watering and grazing areas. The second sample consisted of faecal pats and faeces from wildlife around the pasture. Both types of samples were collected in sterile plastic tubes.

Feedlot operations enrolled in the study were visited five times. On each visit, faeces and environmental samples were collected. The environmental samples included cow feed, water trough sediment and biofilm, feed bunk swabs and pen soil samples. The sampling unit of interest in the feedlot operations was the pen. On each visit, eight samples were collected from different locations within a pen using a conceptual grid sampling to ensure coverage of the pen. Each pen was subdivided into sampling units of nine square feet. A systematic sampling design based on the number of grids in each pen was then employed to collect composite samples of soil. Faecal samples were collected from each pen twice: from animals at an early stage of feeding and at a later stage of feeding. The faecal samples were collected in sterile plastic bottles and stored. A similar number of eight composite soil samples were collected from the same pen using the conceptual grid sampling design described previously to ensure representative sampling. The soil samples were collected in sterile plastic tubes.

Water sampling consisted of composite samples of biofilm swabs of all water troughs in the pen. The composite swabs were transferred to a collection tube and stored at 4°C until shipping. Composite swabs of all feed bunks in the sampled pen were collected in the sampling tubes and handled as described previously. In addition, 200 g of composite feed samples, collected from different spots in the storage area that were identified randomly, was transferred to the sampling tubes.

All samples were stored at 4°C immediately after collection and until shipping the following day through express mail to Cornell for analysis. The commencement of testing was not longer than 72 h after sampling. Samples that took more than 72 h in shipping were not processed.

Detection of *Listeria monocytogenes*

All the samples were processed according to the manufacturer's protocol, BAX System (2009), for detection of

L. monocytogenes, which consisted of a two-stage enrichment followed by DNA detection. Briefly, in the first stage, all samples were pre-enriched with Demi-Fraser broth (Oxoid) at a ratio of 1 : 10 and incubated for 22–26 h at 30°C. In the second stage enrichment, the MOPS (Morpholinepropanesulfonic) Buffered *Listeria* Enrichment Broth – BBL *Listeria* enrichment broth, MOPS free acid and Mops sodium salt (Fisher Scientific, Pittsburgh, PA) were used as selective media in which 0·1 ml of the first stage enrichment was added to 9·9 ml and incubated at 35°C for 18–24 h. After this second enrichment, the samples were heated in a lysis reagent solution. The polymerase chain reaction (PCR) tablets were hydrated with the lysed sample and processed in the automated cycler/detector. The results were displayed by the BAX software (Wilmington, DE) as *L. monocytogenes* positive or negative.

Data analysis

The risk of occurrence of *L. monocytogenes* in each type of sample was computed as the proportion of samples that tested positive out of the samples that were examined using the PCR system. The likelihood of occurrence of *L. monocytogenes* in a particular set of samples in comparison with the other was evaluated using logistic regression analysis, while adjusting for the potential clustering of the results by farm. It was assumed that the unobserved risk factors were randomly distributed among farms, and the overall significance of this assumption was evaluated by using a mixed effects logistic regression model (Rosner *et al.* 1989). The mixed effects logistic regression analysis was performed using the EGRET statistical software (Cytel Statistical Software, Boston, MA, USA). The effect of each factor on the likelihood of infection with the organism was quantified by the odds ratio (OR), which was computed as the exponent of the respective regression coefficient.

Results

Cow-calf operations

A total of 1761 cows were sampled from the 25 ranches with a median number of 70 head per ranch (range 18–139 cows). The occurrence of *L. monocytogenes* among cows appeared to be at a low proportion as the organism was recovered from 3·1% of the sampled cows (Table 1). The organism was detected in cow faecal samples collected from 11 of the 25 operations (herd prevalence 44%). The within-herd prevalence of *L. monocytogenes* ranged from 0% to 29% and the average was 3·4%.

A total of 705 calves were sampled from these ranches with a median number of 28 animals per operation (range 12–60 calves). There was no significant difference in the shedding proportion of *L. monocytogenes* between cows and calves; calves shed the organism at an average rate of 3·7%. The herd prevalence rate for calves was 28%. The within-herd prevalence of *L. monocytogenes* among calves ranged from 0% to 23% and the average was 2·5%.

Table 1 Prevalence of *Listeria monocytogenes* in different samples collected calf-cow and feedlot operations in California

Type of sample	Number of samples	Prevalence (%)	95% Confidence interval
Calf-cow operations			
Cows	1761	3·1	2·2–3·9
Calf	705	3·7	2·3–5·1
Faeces from other animals	40	2·5	0–7·6
Cut-grass	132	5·3	1·4–9·4
Soil	132	5·3	1·4–9·2
Entry point in irrigation ditch	16	0	–
Exist point from irrigation ditch	15	14·3	0–35·3
Water troughs	32	3·1	0–9·5
Water from ponds	31	6·5	0–15·6
Feedlot operations			
Cows	975	0·3	0–0·5
Soil	972	0·7	0·2–1·3
Feed bunk	121	2·5	0–5·3
Water trough	121	0·8	0–2·5
Cow feed	118	1·7	0–4·1

The organism was detected at a relatively low prevalence in samples collected from noncattle faeces (scat from other animals, presumably from wildlife) on the premises (prevalence 2·5%).

Listeria monocytogenes was recovered at a relatively high proportion in environmental samples in comparison with faecal samples. Twenty-eight per cent of the farms had *L. monocytogenes* in at least one of the cut-grass samples collected from the premises. The overall prevalence of the organism in grass cut samples was 5·3%, which is slightly higher that than in animal samples but not significantly different. The within-farm prevalence of the organism was 4% (range 0–33%). The number of composite grass samples collected from each farm ranged from 6 to 12 samples. On the other hand, the organism was detected in at least one of the soil samples from 20% of the ranches in the study. The overall prevalence of *L. monocytogenes* in soil samples was 5·3%, which was similar to that observed for the grass cut samples (Table 1). The within-farm prevalence of the organism was 5·3% (range 0–43%).

Listeria monocytogenes was prevalent in water sources on cow-calf farm operations. Water samples were collected from stream entry and exit points from irrigation

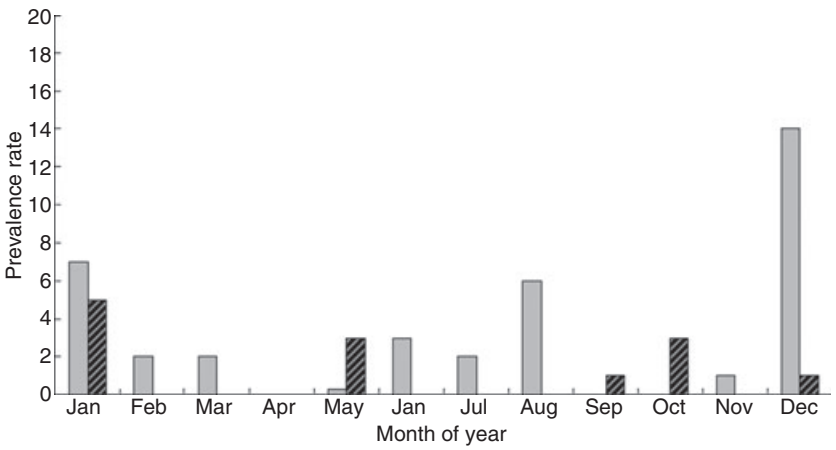

Figure 1 Distribution of monthly occurrence of *Listeria monocytogenes* among calf-cow (□) and feedlot (▨) operations in the study population.

ditches from 12 of the 25 farms enrolled. None of the samples that were collected from the entry point of irrigation ditches were positive for the organism. However, the average recovery rate of *L. monocytogenes* from exit points from the irrigation ditches was 14·3%. The within-farm prevalence of the organism in stream exit samples was 2·6% and the range was 0–50%. These findings imply that most of the contamination in the irrigation ditches happens in the farm, because only exit point samples were positive.

Composite water trough biofilm swabs were collected from 21 farms in the study, and the organism was detected in 3·1% of these samples (Table 1). The number of samples collected per ranch ranged from one to seven composite samples. Samples were also collected from stock ponds in 15 farms, and the organism was detected in two of these farms. The prevalence of *L. monocytogenes* among these samples was 6·5% (Table 1).

Figure 1 shows the monthly distribution of the occurrence of *L. monocytogenes* in the cow-calf population. It appears that the organism is endemic in the farms visited, where it was detected in samples collected from these farms in every month of the year except for April, September and October. Although higher detection rates of the organism were observed in December and January, there was no distinct seasonal pattern of the occurrence of the organism in this population.

Table 2 presents the results of the logistic regression analysis to assess the difference in the occurrence of *L. monocytogenes* in different samples, while controlling for the potential clustering of the prevalence by farm. The likelihood of occurrence of the organism in all sources in the farm was compared to the prevalence in cows (reference category) (Table 2). There was no significant difference in the occurrence *L. monocytogenes* among these sources; however, there was evidence of clustering of the occurrence of the organism by farm as the random effect parameter was significantly different from zero (Table 2).

Table 2 The likelihood of *Listeria monocytogenes* among different samples collected from cow-calf operations while controlling for the potential clustering of the organism by farm

Risk factor	Regression coefficient	Standard error	Odds ratio	95% confidence interval
Referent	−15·058	0·656	–	–
Type of samples				
Cow	0	0	1·0	–
Calf	0·184	0·300	1·2	0·7–2·2
Cut-grass	0·480	0·536	1·6	0·6–4·6
Soil	0·438	0·554	1·6	0·5–4·6
Water samples	0·382	0·509	1·5	0·5–4·0
Random effect parameter	3·867	0·321	–	–

Table 3 Correlation between detecting *Listeria monocytogenes* among different samples collected from cow-calf beef operations

Source of sample	Cow	Calf	Grass	Soil
Cow	–	–	–	–
Calf	0·49	–	–	–
Grass	−0·03	−0·15	–	–
Soil	0·62	0·96	−0·1	–
Ponds	0·62	0·96	−0·1	1·0

Table 3 presents the correlations of the farms' detection rates of *L. monocytogenes* among different samples collected from cow-calf operations. There was relatively high correlation between the presence of the organism in samples collected from either soil or water ponds and faecal samples collected from cows. Because these samples were collected in a cross-sectional study, it is difficult to

conclude whether the cow contracted the infection from these two sources or contaminated them. A similar pattern of association was observed between samples collected from calves and either of these two sources. There was a high association between the presence of *L. monocytogenes* in soil samples and in ponds in the farms. Although there was a correlation between the presence of the organism in samples collected from cows and those collected from calves, the correlation coefficient is not high (Table 3).

Feedlot operations

A total of 2307 samples were collected from 25 feedlot operations in the target population. Table 1 lists the number of samples collected per source and prevalence of *L. monocytogenes* in each type of sample. Samples collected from an average of 40 animals (range 16–48 cows) per operation. The organism was detected in faecal samples in two farms at the rate of 1·6% and 2·1%, respectively.

Soil samples were collected from all feedlot operations in the study at an average of 40 samples per farm (range 16–48). *Listeria monocytogenes* was not common in the soil in these operations because the organism was detected in samples from only one farm at a prevalence of 11%. A total of 64 composite soil samples were collected from that particular farm. Furthermore, *L. monocytogenes* was not common in feed bunk samples from feedlot operations. It was only detected in samples collected from two farms. Within these two farms, the organism was detected in one of the five samples in the first and two of the six samples collected in the second farm. Composite biofilms swabs were collected from water troughs in pens in these operations, and the organism was detected in only one pen in a single farm. Similarly, *L. monocytogenes* was not common in the target population, where it was detected in stored feed that was collected from two farms at 16% and 32%, respectively.

Figure 1 shows the monthly detection rate of *L. monocytogenes* in samples collected from feedlot operations in the sampled populations. The detection rate of the organism varied throughout the year with relatively high rate in January, May and October. There was no apparent clustering of detection by season.

In the final analysis, we compared the likelihood of detecting *L. monocytogenes* in samples collected from feedlot operations to that in samples from cow-calf farms, while controlling for the potential clustering of the occurrence of the organism by premises (Table 4). The likelihood of detecting the organism in samples from cow-calf farms was very high indicating that *L. monocytogenes* was more prevalent in these premises compared to feedlot operations.

Table 4 Association between the type of production (cow-calf *vs* feedlot operations) and the likelihood of *Listeria monocytogenes*

Risk factor	Regression coefficient	Standard error	Odds ratio (95% confidence interval)
Type of production			
Cow-calf	0	–	–
Feedlot	−36·08	03·77	0·03 (0·01–0·07)
Constant	−15·145	0·603	–
Random effect parameter	3·933	0·282	–

Discussion

Very few studies around the world, and none in the United States, have investigated the occurrence of *L. monocytogenes* in beef cattle operations (Young and Firehammer 1958; Hofer 1983; Inoue *et al.* 2000; Bailey *et al.* 2003). However, most of these reports have focused on investigating this pathogen only at meat processing plants (Young and Firehammer 1958; Rivera-Betancourt *et al.* 2004; Madden *et al.* 2007).

The observed shedding rate of 3·1% among cow-calf operations was not substantively different than the 2% rate reported in Australia and the 4·8% reported in Ireland (Bailey *et al.* 2003; Madden *et al.* 2007). However, the latter two rates were reported in faecal samples collected from cows at the processing plants. It should also be pointed out that sampling animals at the abattoirs is likely to result in a higher prevalence because of the stress effect of transportation of cows on the shedding rate (Fenlon *et al.* 1996). Other studies reported higher shedding rates among pastured animals to be processed at the abattoir (Buncic 1991; Bailey *et al.* 2003). We believe that these higher rates are a reflection of the variation in the impact of transportation stress among cows which harboured the organism and the change in the immediate environment in terms of crowding. Hence, animals in our study were sampled at the farm.

The presence of *L. monocytogenes* among beef cattle operations has been incriminated in several cases of abortion (Young and Firehammer 1958). However, none of the farms' owner or manager reported an increased incidence of abortions among the herds surveyed in this study.

Although the organism appears to be common among dairy farms, it was relatively uncommon in calf-cow operations (Skovgaard and Morgen 1988; Hassan *et al.* 2000). Several factors might have contributed to this difference in the shedding rate including the management practices. Historically, one of the incriminated sources of *L. monocytogenes* in dairy farms was contaminated silage;

however, feeding silage is an uncommon practice among calf-cow operations (Gray 1960; Ueno *et al.* 1996).

Apparently *L. monocytogenes* was prevalent among calves in cow-calf operations in our study where the organism was detected in at least one calf in 33% of the farms. This finding is not surprising because the organism is perceived to occur ubiquitously (Gray 1963; Ueno *et al.* 1996); however, the occurrence of the organism was reported to vary by region among beef cattle slaughtered in the United States (Rivera-Betancourt *et al.* 2004). This variation in the occurrence of the organism among farms in the study population could be attributed to several factors including management and geography (Fenlon *et al.* 1996; Ueno *et al.* 1996); however, there was no high correlation between the occurrence of the organism in calves and in cows from the same farm. The detection of *L. monocytogenes* in ponds and soil samples correlated with the detection of the organism in samples collected from calves suggests that either of these could be a source of infection. Because calves are likely to contract the infection through the oral route, it is not unreasonable to incriminate contaminated ponds as a source of infection (Renter *et al.* 2003, 2004; Nightingale *et al.* 2004). Because no *L. monocytogenes* was detected in water samples collected from entry points to irrigation ditches, it is not unreasonable to assume that most of the presence of the organism in water samples could be attributed to the perpetuation of the infection among animals and contamination in the farm. This finding is supported by observation of the presence of the organism at a relatively high rate at exit points from irrigation ditches rather than from entry points to the ditches. Our finding is consistent with results from other studies in which the authors found higher occurrence of *Listeria* in catchments near livestock operations (Lyautey *et al.* 2007).

Listeria monocytogenes was isolated from 5·3% of cut grass and soil samples collected in this study, and there was a high correlation between the occurrences of the pathogens in both samples within the farms. However, there was no correlation between detecting this organism in cattle and in grass, which lead to the speculation that it was unlikely that the animals were exposed to the organism through grass. On the contrary, it was most likely that infected animals had contaminated the grass during grazing. There is supporting evidence in the literature for this observation as *L. monocytogenes* has been shown to survive in grass for more than 4 months (Hutchison *et al.* 2005c). Interestingly enough, there was no correlation between the detection of organisms in grass samples and in either soil or pond samples. Studies on the occurrence of *L. monocytogenes* on grass made a similar conclusion and found that there was a low shedding rate among animals fed largely hay (Fenlon *et al.* 1996; Hutchison *et al.* 2005b). However, there was a high correlation between detecting *L. monocytogenes* in samples collected soil and from ponds. This finding favours the speculation that most of the soil contamination ends in water ponds. However, we caution from the potential of overinterpretation of these finding in this investigation because of the nature of our study, a cross-sectional design. One of the inherited problems in cross-sectional studies is the difficultly of discerning the directionality of the association in the detection of organisms among different samples and hence the causal relationship.

Studies on the prevalence of *Listeria* spp. in dairy farms among animal and environmental samples have described the pattern of occurrence of the organism as seasonal but have not consistently shown whether the organism is more prevalent in winter or summer (Husu 1990; Hassan *et al.* 2000; Hutchison *et al.* 2005b). In this study, which was conducted on beef cattle, we were not able to ascertain any seasonal variation. The highest detection rate of *L. monocytogenes* was observed during the months of December and January, a finding that was consistent with the report that attributing this high rate to the indoor season where animals are crowded together (Husu 1990). The animals sampled in this study are spread out in the pasture the majority of the time and high stocking density is not a factor. It is possible that the lack of crowding explains the lack of seasonal pattern of occurrence of the organism among animals in this study.

It was surprising to find that *L. monocytogenes* was less common in feedlots in comparison with cow-calf operations. This finding is different from the finding in other populations that were investigated at the abattoir level where the authors reported a higher rate among animals originated from feedlot in comparison with pastured beef cattle (Bailey *et al.* 2003). The argument for the expected high prevalence of *L. monocytogenes* among animals acquired from feedlot operations was explained by the feeding of silage (Gray 1960; Fenlon *et al.* 1996; Hassan *et al.* 2001; Bailey *et al.* 2003). However, these outbreaks have been linked primarily to feeding of spoilage silage (Vazquez-Boland *et al.* 1992; Wiedmann *et al.* 1997; Hassan *et al.* 2001; Laven and Lawrence 2006). In our study, the highest detection rate of the organism was found in feed and feed bunk samples in comparison with the animal samples, but there was no correlation between detection of the organism in the feed samples and the animals' faeces. Hence, we do not believe that the feed would play a major role in the introduction of the infection to these operations.

In conclusion, our study showed that *L. monocytogenes* was present in animal and environmental samples collected from cow-calf and feedlot operations at different rates. The organism was detected at a higher rate among

animals in cow-calf farms in comparison with feedlot animals. These results suggest that the presence of the organism at the preharvest level might contribute to the contamination of the beef carcasses and products at the processing plants, and hence put humans at risk of listeriosis. If risk mitigation strategies were to be implemented to reduce the public health risk associated with the consumption of beef and beef products, risk at the preharvest level should not be ignored. This is critical because, unlike in dairy products, there is no intervention in beef products beyond sanitary measures to control and eliminate the hazard of the pathogen in food products.

Acknowledgement

The project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2002-35200-01960.

References

Bailey, G.D., Vanselow, B.A., Hornitzky, M.A., Hum, S.I., Eamens, G.J., Gill, P.A., Walker, K.H. and Cronin, J.P. (2003) A study of the foodborne pathogens: *Campylobacter*, *Listeria* and *Yersinia*, in faeces from slaughter-age cattle and sheep in Australia. *Commun Dis Intell* **27**, 249–257.

Bax System (2009) *PCR Assay for Listeria monocytogenes*. Dupont QualiconProducts. Available at: http://www.2.dupont.com/Qualicon/en_US/products/BAX_System/bax_lmono.html.

Borucki, M.K., Gay, C.C., Reynolds, J., McElwain, K.L., Kim, S.H., Call, D.R. and Knowles, D.P. (2005) Genetic diversity of *Listeria monocytogenes* strains from a high-prevalence dairy farm. *Appl Environ Microbiol* **71**, 5893–5899.

Buncic, S. (1991) The incidence of *Listeria monocytogenes* in slaughtered animals, in meat, and in meat products in Yugoslavia. *Int J Food Microbiol* **12**, 173–180.

Fenlon, D.R., Wilson, J. and Donachie, W. (1996) The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. *J Appl Bacteriol* **81**, 641–650.

Gray, M.L. (1960) A possible link in the relationship between silage feeding and listeriosis. *J Am Vet Med Assoc* **136**, 205–208.

Gray, M.L. (1963) Epidermiological aspects of listeriosis. *Am J Public Health Nations Health* **53**, 554–563.

Gray, M.L., Singh, C. and Thorp, F. Jr (1956) Abortion and pre- or postnatal death of young due to *Listeria monocytogenes* III. Studies in ruminants. *Am J Vet Res* **17**, 510–516.

Gray, M.J., Zadoks, R.N., Fortes, E.D., Dogan, B., Cai, S., Chen, Y., Scott, V.N., Gombas, D.E. *et al.* (2004) *Listeria monocytogenes* isolates from foods and humans form distinct but overlapping populations. *Appl Environ Microbiol* **70**, 5833–5841.

Hassan, L., Mohammed, H.O., McDonough, P.L. and Gonzalez, R.N. (2000) A cross-sectional study on the prevalence of *Listeria monocytogenes* and *Salmonella* in New York dairy herds. *J Dairy Sci* **83**, 2441–2447.

Hassan, L., Mohammed, H.O. and McDonough, P.L. (2001) Farm-management and milking practices associated with the presence of *Listeria monocytogenes* in New York state dairy herds. *Prev Vet Med* **51**, 63–73.

Hathaway, S.C. (1997) Intensive (pasture) beef cattle operations: the perspective of New Zealand. *Rev Sci Tech* **16**, 382–390.

Hofer, E. (1983) Bacteriologic and epidemiologic studies on the occurrence of *Listeria monocytogenes* in healthy cattles. *Zentralbl Bakteriol Mikrobiol Hyg A* **256**, 175–183.

Husu, J.R. (1990) Epidemiological studies on the occurrence of *Listeria monocytogenes* in the faeces of dairy cattle. *Zentralbl Veterinarmed B* **37**, 276–282.

Hutchison, M.L., Walters, L.D., Avery, S.M. and Moore, A. (2005a) Decline of zoonotic agents in livestock waste and bedding heaps. *J Appl Microbiol* **99**, 354–362.

Hutchison, M.L., Walters, L.D., Avery, S.M., Munro, F. and Moore, A. (2005b) Analyses of livestock production, waste storage, and pathogen levels and prevalence in farm manures. *Appl Environ Microbiol* **71**, 1231–1236.

Hutchison, M.L., Walters, L.D., Moore, T., Thomas, D.J. and Avery, S.M. (2005c) Fate of pathogens present in livestock wastes spread onto fescue plots. *Appl Environ Microbiol* **71**, 691–696.

Inoue, S., Nakama, A., Arai, Y., Kokubo, Y., Maruyama, T., Saito, A., Yoshida, T., Terao, M. *et al.* (2000) Prevalence and contamination levels of *Listeria monocytogenes* in retail foods in Japan. *Int J Food Microbiol* **59**, 73–77.

Laven, R.A. and Lawrence, K.R. (2006) An outbreak of iritis and uveitis in dairy cattle at pasture associated with the supplementary feeding of bale age. *N Z Vet J* **54**, 151–152.

Lyautey, E., Lapen, D.R., Wilkes, G., McCleary, K., Pagotto, F., Tyler, K., Hartmann, A., Piveteau, P. *et al.* (2007) Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of the South Nation River Watershed, Ontario, Canada. *Appl Environ Microbiol* **73**, 5401–5410.

Madden, R.H., Murray, K.A. and Gilmour, A. (2007) Carriage of four bacterial pathogens by beef cattle in Northern Ireland at time of slaughter. *Lett Appl Microbiol* **44**, 115–119.

Nightingale, K.K., Schukken, Y.H., Nightingale, C.R., Fortes, E.D., Ho, A.J., Her, Z., Grohn, Y.T., McDonough, P.L. *et al.* (2004) Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Appl Environ Microbiol* **70**, 4458–4467.

Norton, D.M. and Braden, C.R. (2007) Foodborne listeriosis. In *Listeria, Listeriosis, and Food Safety* ed. Ryser, E.T. and Marth, E. pp. 305–356. Boca Raton, FL: CRC press.

Renter, D.G., Sargeant, J.M., Oberst, R.D. and Samadpour, M. (2003) Diversity, frequency, and persistence of *Escherichia*

coli O157 strains from range cattle environments. *Appl Environ Microbiol* **69**, 542–547.

Renter, D.G., Sargeant, J.M. and Hungerford, L.L. (2004) Distribution of *Escherichia coli* O157:H7 within and among cattle operations in pasture-based agricultural areas. *Am J Vet Res* **65**, 1367–1376.

Rivera-Betancourt, M., Shackelford, S.D., Arthur, T.M., Westmoreland, K.E., Bellinger, G., Rossman, M., Reagan, J.O. and Koohmaraie, M. (2004) Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in two geographically distant commercial beef processing plants in the United States. *J Food Prot* **67**, 295–302.

Rosner, B., Willett, W.C. and Spiegelman, D. (1989) Correction of logistic regression relative risk estimates and confidence intervals for systematic within-person measurement error. *Stat Med* **8**, 1051–1069.

Schwartz, B., Ciesielski, C.A., Broome, C.V., Gaventa, S., Brown, G.R., Gellin, B.G., Hightower, A.W. and Mascola, L. (1988) Association of sporadic listeriosis with consumption of uncooked hot dogs and undercooked chicken. *Lancet* **2**, 779–782.

Skovgaard, N. and Morgen, C.A. (1988) Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. *Int J Food Microbiol* **6**, 229–242.

Stockton, J.J., Neu, L., Carpenter, W.S. and Gray, M.L. (1954) The association of *Listeria monocytogenes* with abortion; review of the literature and report of a bovine case. *J Am Vet Med Assoc* **124**, 102–105.

Troutt, H.F. and Osburn, B.I. (1997) Meat from dairy cows: possible microbiological hazards and risks. *Rev Sci Tech* **16**, 405–414.

Ueno, H., Yokota, K., Arai, T., Muramatsu, Y., Taniyama, H., Iida, T. and Morita, C. (1996) The prevalence of *Listeria monocytogenes* in the environment of dairy farms. *Microbiol Immunol* **40**, 121–124.

Vazquez-Boland, J.A., Dominguez, L., Blanco, M., Rocourt, J., Fernandez-Garayzabal, J.F., Gutierrez, C.B., Tascon, R.I. and Rodriguez-Ferri, E.F. (1992) Epidemiologic investigation of a silage-associated epizootic of ovine listeric encephalitis, using a new *Listeria*-selective enumeration medium and phage typing. *Am J Vet Res* **53**, 368–371.

Wiedmann, M., Arvik, T., Bruce, J.L., Neubauer, J., del Piero, F., Smith, M.C., Hurley, J., Mohammed, H.O. *et al.* (1997) Investigation of a listeriosis epizootic in sheep in New York state. *Am J Vet Res* **58**, 733–737.

Young, S. and Firehammer, B.D. (1958) Abortion attributed to *Listeria monocytogenes* infection in a range herd of beef cattle. *J Am Vet Med Assoc* **132**, 434–438.