APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1988, p. 497–501
0099-2240/88/020497-05$02.00/0

Vol. 54, No. 2

# Fate of *Listeria monocytogenes* in Tissues of Experimentally Infected Cattle and in Hard Salami

JENNIFER L. JOHNSON,[1,2] MICHAEL P. DOYLE,[1]* ROBERT G. CASSENS,[2] AND JEAN L. SCHOENI[1]

*Food Research Institute[1] and Department of Meat and Animal Science,[2] University of Wisconsin-Madison, Madison, Wisconsin 53706*

Received 21 September 1987/Accepted 16 November 1987

**Muscle, organ, and lymphoid tissues of four Holstein cows experimentally inoculated (intravenously) with *Listeria monocytogenes* were examined 2, 6, or 54 days postinoculation for the presence of the organism by direct plating and cold enrichment procedures. *L. monocytogenes* was isolated from 66% of the tissues sampled; 38% of the isolations were attributed to the use of cold enrichment. Isolation of the organism from muscle tissue was possible only with animals inoculated 2 days before slaughter. The fate of *L. monocytogenes* during the manufacture and storage of fermented hard salami made from this meat also was determined. Three sausage treatments were evaluated: (i) uninoculated control sausage, (ii) "naturally" contaminated sausage (NC) made from meat of an experimentally inoculated cow, and (iii) sausage made from beef inoculated with a laboratory culture of *L. monocytogenes* (I). Initial *Listeria* levels in NC and I sausage were $10^3$ CFU/g in trial 1 and $10^4$ CFU/g in trial 2. Numbers of *L. monocytogenes* decreased by approximately 1 $\log_{10}$ CFU/g during fermentation and decreased further during drying and refrigerated storage. Small numbers (≤20 CFU/g) of *L. monocytogenes* were present in I and NC sausage at the end of 12 weeks of refrigerated storage; recovery of these organisms generally depended on the use of an enrichment procedure. The results indicate that *L. monocytogenes* does not multiply during the fermentation and drying processes typical of hard salami manufacture but that survival may occur if the organism is initially present at ≥$10^3$ CFU/g.**

The role of *Listeria monocytogenes* as an important food-borne pathogen is now firmly established. Outbreaks of food-borne listeriosis have been linked to the consumption of milk, cheese, and cole slaw, but presently there is no direct evidence for transmission of human listeriosis by meat.

Minced meat from a dead calf was suspected of transmitting the organism to the wife of a Dutch farmer in the early 1960s; this could not be confirmed because the remainder of the meat was sterilized during canning (11). Meat was also suspected of transmitting *L. monocytogenes* to four people living in a 3-block area of Uppsala, Sweden. The organism could not be recovered from milk, water, meat, or vegetable supplies, and only the meat was supplied by a common source (16).

While there is no doubt that *L. monocytogenes* can be found on meat and meat products (18), the extent of contamination is not known. Estimates of contamination with *L. monocytogenes* in Europe have been placed at 6 to 12% for raw delicatessen products and processed meats and at up to 33% for dry sausage (8,15), based on limited numbers of samples. Carcass contamination is reported to be lower (15), and Cottin et al. (5) were unable to isolate *L. monocytogenes* from the muscle of 514 cattle, although the organism was recovered from the spleen or lungs of 3% of the cattle. The purpose of this study was to examine the extent of *L. monocytogenes* contamination of tissues from four experimentally infected cows and to determine the fate of the organism in sausage made from contaminated muscle tissue.

## MATERIALS AND METHODS

**Inoculation of cows and slaughtering process.** Four Holstein cows (3 to 6 years of age) were used in this study; the animals had previously been inoculated (intramammary) as part of an experiment examining the behavior of *L. monocytogenes* during milk pasteurization (7). All cows had been immunocompromised with dexamethasone for 5 days at the end of the pasteurization study. Cow A was not reinoculated with *L. monocytogenes* before slaughter; 54 days had elapsed since the previous inoculation. Cow B received $5 \times 10^{10}$ *L. monocytogenes* Scott A intravenously (i.v.) 6 days before slaughter. Cows C and D were given $4.3 \times 10^{10}$ and $4.8 \times 10^{11}$ organisms (i.v.), respectively, 2 days before slaughter.

At slaughter, animals were stunned with a captive-bolt gun and then exsanguinated in the necropsy room of the University of Wisconsin School of Veterinary Medicine; only one animal was killed at a time. Hides were removed from the carcasses with knives sterilized in 95% ethanol; external fat and superficial muscles were similarly removed and discarded. Deep muscle from the chuck-rib portions (front shoulder, brisket, and rib region anterior to the sixth rib), loin portions, plate-flank portions (abdominal muscles and flank), and round portions (hind leg) was then excised, and the meat was placed in sterile plastic bags, refrigerated for 1 day, and then frozen at −20°C. Duplicate 50- to 100-g composite muscle samples from each portion were taken to be assayed for *L. monocytogenes*. Samples (ca. 100 g) were also taken in duplicate from the medulla, anterior spinal cord, mammary gland tissue, mesenteric and mammary lymph nodes, spleen, kidney, tongue, heart, liver, blood, and feces.

**Sampling of bovine tissues and confirmation of isolates.** Samples weighing 15 to 25 g were diluted 1:10 in tryptose broth (Difco Laboratories, Detroit, Mich.) and homogenized for 2 min in sterile blender jars on a Waring blender. Portions of this homogenate were serially diluted in 0.01 M phosphate-buffered saline solution (PBS), and 0.1-ml portions were spread plated onto duplicate plates of McBride *Listeria* agar (14). Plates were incubated at 37°C for 48 h under

* Corresponding author.

microaerobic conditions (5% $O_2$, 10% $CO_2$, and 85% $N_2$). The remaining homogenate was poured into sterile plastic bags and put into cold enrichment at 4°C. Cold-enriched sample homogenates were sampled at 1, 2, 3, 4, 6, and 8 weeks by spread plating 0.1-ml portions onto duplicate McBride agar plates; the plates were incubated as described above.

From each pair of plates, five colonies typical of *L. monocytogenes* (bluish, translucent, slightly beta-hemolytic, and 0.5 to 1.5 mm in diameter) were chosen for confirmation. Isolates were streaked onto tryptose agar (Difco), tested for catalase production, and then confirmed with the following tests: tumbling motility, umbrella motility when grown on motility agar at 25 and 37°C, carbohydrate fermentation (salicin, rhamnose, maltose, dextrose, dulcitol, mannitol, and xylose), nitrate reduction, litmus milk reduction, methyl red reaction, blackening of esculin broth, Gram stain, and serology with *Listeria* type 4 O antiserum (Difco). The pathogenicity of *L. monocytogenes* isolates was determined by injecting (intraperitoneally) a 24-h culture of ca. $10^9$ washed cells suspended in PBS into each of five mice (ICR females, 16 to 20 g; Harlan Sprague-Dawley, Indianapolis, Ind.) and observing the mice for 7 days.

**Sausage manufacture.** Three sausage treatments were examined: (i) uninoculated control sausage (C); (ii) inoculated sausage (I), consisting of beef trimmings inoculated with a laboratory culture of *L. monocytogenes* Scott A; and (iii) "naturally" contaminated sausage (NC) made from the meat of an experimentally infected cow. For trial 1, one batch of each type of sausage was made. NC sausage from this trial was excessively lean (4% fat), and because this is not representative of common commercial salami, the decision was made to add exogenous fat to the NC sausage made in trial 2. To determine what effect, if any, fat would have on the survival of *L. monocytogenes*, two batches of NC sausage with 10% added fat (NC-F) were included in trial 2. Data from NC-F sausage are reported as an average of values from the two batches.

Inoculum for the I sausage was prepared from a culture of *L. monocytogenes* Scott A grown aerobically in tryptose broth for 18 h at 37°C with agitation. The culture (50 ml) was centrifuged at 6,000 × *g* for 15 min, and the pellet was suspended in 10 ml of 0.01 M PBS. The $A_{500}$ of the cell suspension was read, and the suspension was diluted appropriately in PBS. The inoculum level was adjusted to approximate the level found in NC meat; for trial 1, this was $10^3$ CFU/g, and for trial 2, it was $10^4$ CFU/g. The inoculum was then added to 190 ml of PBS for distribution into 13.6 kg of coarsely ground meat. Enumeration was done by spread plating 0.1 ml of serial (1:10) dilutions of the inoculum onto duplicate tryptose agar plates and incubating the plates at 37°C for 48 h.

Beef trimmings used for the C and I sausage were obtained from the University of Wisconsin-Madison Muscle Biology Laboratory. NC meat was obtained from cow D as described above. All beef had been stored at −20°C before use and was thawed at 4°C for 3 days before sausage manufacture.

For I, C, and trial 1 NC batches of sausage, 13.6 kg of cold beef trim was ground through a 0.95-cm (3/8-in.) plate on a Hobart laboratory grinder (model 84142; Hobart Manufacturing Co, Troy, Ohio); 12.3 kg of NC meat was ground for each of the two NC-F batches in trial 2. Ground meat was then added to a Buffalo model 2VSS mixer (John E. Smith's Sons Co., Buffalo, N.Y.) and mixed for 4 min with inoculum (I batches) or 200 ml of PBS (C, NC, and NC-F batches). At this time, trial 2 NC-F batches received 1.4 kg of coarsely ground beef fat obtained from a local supermarket. Glucose (85 g), commercial spice premix (407 g), and a commercial cure mixture (253 g, formulated for 156 ppm [156 μg/g] $NaNO_2$ and 3.3% NaCl) were added and mixed for 3 min. Finally, 70 g of resuspended glucose-fermenting *Pediococcus acidilactici* sausage starter culture (Lactacel 115; Microlife Technics, Sarasota, Fla.) was added and mixed for 4 min. The sausage batter was then reground through a 0.48-cm (3/16-in.) grinder plate and stuffed into 5.08-cm (2-in.)-diameter protein-coated fibrous casings (Viskase Corp., Chicago, Ill.) with a hand stuffer (F. Dick; Koch Supplies, Inc., Kansas City, Mo.). Sausages were then tied by hand. The finished sausages were approximately 27 to 28 cm long and weighed about 500 g. The sausage-processing equipment was washed and sanitized with Antibac B solution (Diversey Wyandotte, Wyandotte, Mich.) between batches.

Sausages were brought to the University of Wisconsin-Madison Biotron facility and hung on metal racks in an environmental chamber for fermentation and drying. Fermentation was at 40°C and 85% relative humidity for 24 h and was followed by drying at 13°C, 67% relative humidity, and an airflow of 25 ft/min (762 cm/min) for 9 days. Dried sausages were then taken to the Food Research Institute and individually vacuum packaged in gas-impermeable Curlon bags (nylon-Saran-polyethylene; $O_2$ transmission of 0.8 to 1.0 $cm^3/645\ cm^2$ per 24 h at 22.8°C; $CO_2$ transmission of 2.5 to 3.0 $cm^3/645\ cm^2$ per 24 h at 22.8°C; $H_2O$ transmission of 0.5 $g/645\ cm^2$ per 24 h at 37.8°C and 90% relative humidity; Curwood, Inc., New London, Wis.) by using a Multivac AGW vacuum packager (Sepp Haggenmüller KG, Wolfertschwenden, Federal Republic of Germany). Packaged sausages were held at 4°C until sampled.

**Sausage sampling and confirmation of *Listeria* isolates.** Mean from each treatment (C, I, NC, and NC-F) was sampled after coarse grinding by placing a 25-g composite sample (from different locations within the meat mass) in a sterile stomacher bag (Tekmar Co., Cincinnati, Ohio), adding 225 ml of *Listeria* enrichment broth (6), and macerating for 2 min in a stomacher (model 400; Tekmar Co.). The homogenized samples were serially diluted (1:10) in PBS, and 0.1-ml portions were spread plated in duplicate onto LPM agar (13); the plates were incubated at 30°C for 5 days. The original homogenates were also enriched at 30°C for 15 to 24 h according to the method of the Microbiology Division, Food Safety and Inspection Service, U.S. Department of Agriculture, Beltsville, Md. I, NC, and NC-F sausages were sampled at the time of manufacture and at 0.5, 1, 3, 6, and 10 days and 2, 3, 4, 6, 8, 10, and 12 weeks after manufacture. C sausages were sampled at manufacture and at 1, 6, and 10 days and 4, 8, and 12 weeks. Two sausages of each type were sampled at each time.

For confirmation, colonies which were about 1 mm in diameter and translucent gray-white on LPM agar were streaked onto tryptose agar and confirmed as described above. Slide serology with *Listeria* type 1 and type 4 O antisera (Difco) was done, and the pathogenicity of *Listeria* isolates was determined as described above.

**pH determinations and chemical analyses.** For pH determination, a 10-g sample was taken from each sausage, combined with 90 ml of distilled, deionized water, and macerated for 2 min by a stomacher; the pH was then determined by a combination electrode (Corning Glass Works, Corning, N.Y.). Moisture, fat, Kjeldahl protein, and salt determinations were done on finished sausages according to the procedures of the Association of Official Analytical Chem-

ists (1). Water activity ($a_w$) was also determined with a Hygroline $a_w$ instrument (Beckman Instruments, Inc., Fullerton, Calif.).

## RESULTS

**Bovine tissue experiment.** The results of assay of the various tissues for *L. monocytogenes* are shown in Table 1. The organism was isolated from eight tissues and from the feces of cow A; seven of the isolations were by enrichment, and two were by direct plating. Cow B was reinoculated with *L. monocytogenes* 6 days before slaughter, and *L. monocytogenes* was recovered twice by enrichment and once by direct plating. Tissues of cow C, an animal which was reinoculated 2 days before slaughter, yielded *L. monocytogenes* 4 times by enrichment and 10 times by direct plating. Cow D, also reinoculated 2 days before slaughter, had *L. monocytogenes* in every tissue examined as well as in the feces; 13 isolations were made by direct plating, and 3 were made by enrichment.

Recovery of *L. monocytogenes* varied between animals and was affected by the amount of time between inoculation and slaughter. Cows C and D, inoculated 2 days before slaughter, had the highest rate of recovery of *L. monocytogenes*. The organism was always isolated from mammary gland tissue and mammary lymph nodes and usually was isolated from the heart, liver, and spleen. With the exception of cow B, *L. monocytogenes* was recovered by direct plating of mesenteric lymph nodes and kidney samples from all animals. Overall, the organism was recovered from 66% of the tissue and fecal samples examined; all isolates were serotype 4, i.e., the same serotype as strain Scott A. Cold enrichment was responsible for 38% of the isolations.

TABLE 1. Isolation of *L. monocytogenes* from tissues of four experimentally infected cows

| Specimen | *L. monocytogenes* ($\log_{10}$ CFU/g) recovered from cow[a]: | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Feces | E[b]-8 | ND[c] | ND | E-4 |
| Blood | ND | ND | 2.75[d] | 2.82[d] |
| Brain | E-4 | ND | ND | E-8 |
| Spinal cord | ND | ND | E-2 | E-2 |
| Mammary gland | E-1 | E-2 | 3.20[d] | 2.33[d] |
| Mesenteric lymph node | 2.83[e] | ND | 2.76[e] | 1.30[e] |
| Mammary lymph node | E-6 | 2.15[e] | 3.09[d] | 3.03[d] |
| Spleen | E-1 | ND | 4.67[d] | 4.81[d] |
| Kidney | 2.56[d] | ND | 2.43[d] | 3.81[d] |
| Tongue | ND | ND | E-2 | 2.38[d] |
| Heart | E-1 | ND | 2.00[e] | 2.45[d] |
| Liver | E-3 | ND | 4.03[d] | 4.74[d] |
| Chuck-rib | ND | ND | 1.85[e] | 2.32[e] |
| Loin | ND | ND | E-2 | 2.25[d] |
| Plate-flank | ND | E-2 | 2.10[e] | 2.41[d] |
| Round | ND | ND | E-3 | 2.45[d] |
| % Recovery from tissues and feces | 56 | 19 | 88 | 100 |

[a] Cow B received $5 \times 10^{10}$ *L. monocytogenes* i.v. 6 days before slaughter; cows C and D received $4.3 \times 10^{10}$ and $4.8 \times 10^{11}$ *L. monocytogenes*, respectively, i.v. 2 days before slaughter.

[b] E, *L. monocytogenes* (<10 CFU/g) recovered by cold enrichment at week indicated.

[c] ND, None detected.

[d] Mean $\log_{10}$ CFU per gram based on two samples, with duplicate plates per sample.

[e] Mean $\log_{10}$ CFU per gram based on one sample, with duplicate plates per sample.

TABLE 2. *L. monocytogenes* recovered from hard salami during fermentation and storage

| Sampling time[a] | *L. monocytogenes* ($\log_{10}$ CFU/g)[b] recovered | | | |
|---|---|---|---|---|
| | Trial 1[c] | | Trial 2 | |
| | NC | I | NC-F[d] | I[c] |
| Day 0 | 2.94 | 2.86 | 3.96 | 4.15 |
| Day 0.5 | 2.23 | E[e] | 3.77 | 3.40 |
| Day 1 | 1.40 | 1.30 | 2.15 | 2.98 |
| Day 3 | 2.04 | 1.30 | 2.28 | 1.65 |
| Day 6 | 1.30 | E | 1.85 | 1.81 |
| Day 10 | E | 1.70 | 2.32 | 1.30 |
| Week 2 | 1.54 | E | 1.00 | E |
| Week 3 | E | 1.48 | 1.30 | E |
| Week 4 | 1.70 | ND[f] | E | E |
| Week 6 | 1.00 | E | 1.00 | E |
| Week 8 | E | ND | 1.00 | E |
| Week 10 | E | ND | ND | E |
| Week 12 | E | E | 1.30 | E |

[a] Days 0 and 0.5 represent fermentation; days 1, 3, 6, and 10 represent the drying period; and weeks 2, 3, 4, 6, 8, 10, and 12 represent refrigerated storage.

[b] *L. monocytogenes* was recovered by enrichment from C meat in both trials and from one C sausage sampled on day 10 of trial 2.

[c] Values reported are means from two sausages, with duplicate plates per sausage.

[d] Values are means from four sausages representing two batches, with duplicate plates per sausage.

[e] E, *L. monocytogenes* (<10 CFU/g) was recovered only by enrichment.

[f] ND, None detected.

An important observation made during the course of this study is that the number of *L. monocytogenes* recoverable from the muscle of cow D increased by 1 $\log_{10}$/g or more between the time the muscle was initially sampled and the sampling just before sausage manufacture. This meat had been frozen at −20°C for 6 months (sausage made in trial 1) and 8 months (trial 2). Taking into account the proportion of meat derived from each region of the carcass, the meat used in trial 1 should have had $3.3 \times 10^2$ CFU/g, based on the number of *L. monocytogenes* found just after slaughter. Sampling before sausage manufacture revealed $2.2 \times 10^3$ CFU/g. The meat used in trial 2 had $2.2 \times 10^2$ CFU/g initially and yielded $1.1 \times 10^4$ CFU/g when sampled before sausage making.

**Sausage experiment.** *L. monocytogenes* was not recovered by direct plating of control meat or C sausage. However, the organism was present at <10 CFU/g in both batches of control meat and in one C sausage sampled on day 10; isolation of *L. monocytogenes* in these instances was dependent on the use of enrichment procedures. Likewise, *L. monocytogenes* was isolated from enrichment of the fat added to NC-F meat in trial 2. Isolates from control meat in trial 1, C sausage, and fat were all *L. monocytogenes* serotype 4. Serotype 1, xylose-fermenting listeriae were isolated from the control meat used in trial 2. Organisms isolated from the fat and the serotype 1 *Listeria* organism were nonpathogenic to mice.

During fermentation, numbers of *L. monocytogenes* decreased by more than 1 $\log_{10}$ CFU/g in NC, NC-F, and I sausages (Table 2). Numbers of *L. monocytogenes* then remained approximately constant before decreasing to <50 CFU/g by 3 weeks after manufacture.

During further refrigerated storage, *L. monocytogenes* persisted at very low levels and could generally be recovered by enrichment. By 8 weeks after manufacture, the number of *L. monocytogenes* had again decreased, and on several

occasions the organism was not detectable. Survival of listeriae was similar in NC and I sausage, though the organism was more commonly isolated from NC sausage during the last month of storage. Addition of exogenous fat to the NC meat used in trial 2 did not appear to influence survival of *L. monocytogenes*, and the results from trials 1 and 2 closely paralleled each other.

The pH of the sausage decreased from 5.5 (NC meat, trials 1 and 2) and 5.8 (C and I meat, trials 1 and 2) to approximately 4.5 in trial 1 and 4.3 in trial 2 as a result of fermentation. pH values then remained at 4.5 and 4.3 during the drying period and throughout the refrigerated-storage period.

The proximate composition, salt content, and $a_w$ values are shown in Table 3.

## DISCUSSION

To a large extent, recovery of *L. monocytogenes* from bovine tissues, blood, and feces was dependent on the length of time between inoculation and slaughter. This finding is similar to that of Wilkinson and Hall (23) who demonstrated that the degree of difficulty in isolating the organism from experimentally infected mice was directly proportional to the amount of time postinjection. Variability between animals also influenced the extent of recovery of *L. monocytogenes*. Cow A had a relatively high rate of carriage, considering that it had been almost 2 months since the last inoculation. Recovery of the organism from cow B, on the other hand, was poor in spite of reinoculation 6 days before slaughter; this may be indicative of a heightened immune response.

Isolation of *L. monocytogenes* from the lungs, liver, spleen, and kidney of experimentally infected animals seems to be common (12, 23). From pigs and lambs which died of natural listeric infection, Slivko (21) recovered the organism from the brain, spleen, liver, kidney, heart, and lymph nodes but was unable to isolate it from muscle. This may also be true of animals that recover from listeric infection, because the description fits the isolation pattern observed with tissues of cow A. In this experiment, *L. monocytogenes* could be isolated only from the muscle of animals which had been reinoculated 2 days before slaughter; even then, numbers of the organism were several orders of magnitude lower than levels found in the liver and spleen. Use of these organs as food may constitute the greatest exposure to *L. monocytogenes* provided by any body tissue. Examination of mammary tissue and lymph nodes always revealed the presence of the organism, as would be expected given that the animals were inoculated intramammary in an earlier study. The presence of large numbers of *L. monocytogenes* in the lymph nodes is noteworthy simply because these "glands" may be in meat trimmings and, hence, may contaminate meat which was otherwise free of the organism.

TABLE 3. Proximate composition, $a_w$, and NaCl content of hard salami

| Sausage treatment[a] | Protein (%) | Moisture (%) | Fat (%) | MPR[b] | $a_w$ | NaCl (%) |
|---|---|---|---|---|---|---|
| Trial 1[c] | | | | | | |
| C | 29 | 39 | 24 | 1.30 | 0.86 | 5.7 |
| NC | 45 | 37 | 4 | 0.80 | 0.80 | 7.8 |
| I | 25 | 35 | 35 | 1.40 | 0.86 | 5.0 |
| Trial 2 | | | | | | |
| C[c] | 33 | 36 | 17 | 1.10 | 0.81 | 6.2 |
| NC-F[d] | 37 | 35 | 17 | 0.95 | 0.81 | 5.9 |
| I[c] | 35 | 36 | 19 | 1.00 | 0.79 | 5.6 |

[a] Sausages were sampled at the end of the drying period, before being packaged.
[b] MPR, Moisture/protein ratio.
[c] Mean values from triplicate determinations.
[d] Mean values from six determinations (three determinations from each of two batches).

The results of this experiment again prove the value of the cold enrichment procedure advocated by Gray et al. (10). Cold enrichment accounted for recovery of *L. monocytogenes* in 38% of the instances in which the organism was recovered.

An increase in the number of *L. monocytogenes* recoverable after frozen storage has also been observed by Zink et al. (24). Increased recovery may be due to freeze-thaw damage of cell membranes, resulting in the liberation of formerly intracellular organisms. Hence, the use of frozen and thawed meat in sausage manufacture may result in increased numbers of *L. monocytogenes* in the sausage when compared with numbers found in the meat before freezing. While *L. monocytogenes* may grow at 3°C (22), it is unlikely that substantial growth occurred during the 3-day thawing period in a 4°C cooler. Indeed, even after 3 days at 4°C, the majority of the meat still contained ice crystals, and semifrozen meat pieces had to be pried apart before being ground. The organism survived frozen storage at −23°C for up to 20 days (19) and storage at −20°C for approximately 1 year (17).

The results of the sausage experiment failed to demonstrate growth and multiplication of *L. monocytogenes* during the fermentation and drying processes typical of hard salami manufacture. However, the organism did survive such processing, although in decreased numbers. Likewise, survival occurred during a 3-month period of refrigerated storage.

The $a_w$ of the product ranged from 0.79 to 0.86, values considerably lower than the minimum value of 0.90 to 0.91 required for growth of most bacteria (3) and lower than the $a_w$ of 0.91 found in some commercial hard salami. It is possible that *L. monocytogenes* could survive more readily at the higher $a_w$ of commercial sausage, but growth would still seem unlikely given the presence of salt and sodium nitrite, combined with a low pH and low storage temperature. The sausage had a moisture/protein ratio lower than the 1.9:1 value commonly found with hard salami, but moisture contents of 35 to 39% are typical, falling within the range of 25 to 45% reported by Bacus (2).

There is little information available regarding the growth or survival of *L. monocytogenes* in sausage. Studies using a broth system (20) revealed that the organism cannot grow at 4°C and a pH of 5.0 or less. The same authors observed that *L. monocytogenes* could grow in the presence of 25,000 ppm sodium nitrite, and they suggested that sodium nitrite would exhibit an antimicrobial effect only at refrigeration temperatures in products with ≥3.3% NaCl and a pH of ≤5.5. Conner et al. (4) observed that the organism grew at 5°C in cabbage juice containing 5% NaCl but that the number of viable *L. monocytogenes* decreased over time. The organism grew at approximately pH 5.0 but showed little adaptation to low-pH environments. Hence, it appears that growth of *L. monocytogenes* is suppressed in fermented dry sausage, probably by a combination of low pH, elevated sodium chloride levels, the presence of sodium nitrite, and low $a_w$. However, the organism is capable of surviving such conditions.

The recovery of *L. monocytogenes* from enrichments of

uninoculated control meat in both trials and of fat added to NC meat in trial 2 supports the conclusion that the organism is a common contaminant of meat and meat products. In a survey in France of 157 samples of frozen ground beef, lamb, and raw delicatessen meats to be cooked before consumption, Nicolas (15) isolated 10 pathogenic and 14 nonpathogenic strains of *Listeria* spp. Other studies revealed that 19% of the beef, 17% of the pork, and 70% of the poultry entering a Czechoslovakian household in 1 year was contaminated with *L. monocytogenes* (9). Considerably more research needs to be done to determine what role meat and meat products may play in transmission of foodborne listeriosis. Effective measures to control the organism must be developed, together with better and more efficient isolation procedures.

## ACKNOWLEDGMENTS

We thank Ronald Schultz, Gary Garcia, and Peter Maney for assistance during the course of the cow study. We also thank John Cerveny, Oscar Mayer Foods Corp., and Chuck Baum (Biotron) for their help during the sausage experiment, and Gary Gehrke and Curwood, Inc., for donating packaging materials.

This work was supported by the College of Agriculture and Life Sciences, University of Wisconsin-Madison, and by contributions to the Food Research Institute.

## LITERATURE CITED

1. **Association of Official Analytical Chemists.** 1984. Official methods of analysis, 14th ed., p. 431–435. Association of Official Analytical Chemists, Arlington, Va.
2. **Bacus, J.** 1984. Utilization of microorganisms in meat processing, a handbook for meat plant operators, p. 15–18. Research Studies Press, Ltd., Letchworth, England.
3. **Banwart, G. J.** 1981. Basic food microbiology, p. 80. AVI Publishing Co., Westport, Conn.
4. **Conner, D. E., R. E. Brackett, and L. R. Beuchat.** 1986. Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl. Environ. Microbiol. **52:** 59–63.
5. **Cottin, J., H. Genthon, C. Bizon, and B. Carbonnelle.** 1985. Recherche de *Listeria monocytogenes* dans des viandes prélevées sur 514 bovins. Sci. Aliments **5:**145–149.
6. **Donnelly, C. W., and G. J. Baigent.** 1986. Method for flow cytometric detection of *Listeria monocytogenes* in milk. Appl. Environ. Microbiol. **52:**689–695.
7. **Doyle, M. P., K. A. Glass, J. T. Beery, G. A. Garcia, D. J. Pollard, and R. D. Schultz.** 1987. Survival of *Listeria monocytogenes* in milk during high-temperature, short-time pasteurization. Appl. Environ. Microbiol. **53:**1433–1438.
8. **Durst, J., and G. Berencsi.** 1975. Data about listeriosis in Hungary, p. 106–111. *In* M. Woodbine (ed.), Problems of listeriosis. Leicester University Press, Leicester, England.
9. **Elischerová, K., S. Stúpalová, and J. Stepánek.** 1979. Some ecological aspects of *Listeria monocytogenes* in meat industry, p. 148–155. *In* I. Ivanov (ed.), Problems of listeriosis. National Agro-Industrial Union Center for Scientific Information, Sofia, Bulgaria.
10. **Gray, M. L., H. J. Stafseth, F. Thorp, Jr., L. B. Sholl, and W. F. Riley, Jr.** 1948. A new technique for isolating *Listerellae* from the bovine brain. J. Bacteriol. **55:**471–476.
11. **Kampelmacher, E. H.** 1962. Animal products as a source of listeria infections in man, p. 146–156. *In* M. L. Gray (ed.), Second Symposium on Listeria Infection. Montana State College, Bozeman.
12. **Kautter, D. A., S. J. Silverman, W. G. Roessler, and J. F. Drawdy.** 1963. Virulence of *Listeria monocytogenes* for experimental animals. J. Infect. Dis. **112:**167–180.
13. **Lee, W. H., and D. McClain.** 1986. Improved *Listeria monocytogenes* selective agar. Appl. Environ. Microbiol. **52:**1215–1217.
14. **McBride, M. E., and K. F. Girard.** 1960. A selective method for the isolation of *Listeria monocytogenes* from mixed bacterial populations. Lab. Clin. Med. **55:**153–157.
15. **Nicolas, J. A.** 1985. Contamination des viandes et des produits de charcuterie par *Listeria monocytogenes* en Haute-Vienne. Sci. Aliments **5:**175–180.
16. **Olding, L., and L. Philipson.** 1960. Two cases of listeriosis in the newborn, associated with placental infection. Acta Pathol. Microbiol. Scand. **48:**24–30.
17. **Pittman, B., and W. B. Cherry.** 1967. Isolation of *Listeria monocytogenes* from brains of rabies-negative animals. Am. J. Vet. Res. **28:**779–785.
18. **Ralovich, B.** 1984. Listeriosis research, present situation and perspective. Akademia Kiado, Budapest, Hungary.
19. **Selivanov, A. V., and G. A. Grinitsuina.** 1958. Survival of *Listeria* in meat. Veterinariya (Moscow) **35**(6):37–38.
20. **Shahamat, M., A. Seaman, and M. Woodbine.** 1980. Influence of sodium chloride, pH and temperature on the inhibitory activity of sodium nitrite on *Listeria monocytogenes*, p. 227–237. *In* G. W. Gould and J. E. L. Corry (ed.), Microbial growth and survival in extremes of environment. Academic Press, Inc., New York.
21. **Slivko, V. V.** 1958. Survival of *Listeria* in meat. Veterinariya (Moscow) **35**(6):39–40.
22. **Wilkins, P. O., R. Bourgeois, and R. G. E. Murray.** 1972. Psychrotrophic properties of *Listeria monocytogenes*. Can. J. Microbiol. **18:**543–551.
23. **Wilkinson, T. R., and E. R. Hall.** 1971. Rapid method for the isolation of *Listeria monocytogenes* from experimentally infected mice. Appl. Microbiol. **12:**112–118.
24. **Zink, A., G. C. deMello, and R. L. Burkhart.** 1951. Listeriosis—field and laboratory studies, and aureomycin activity. Am. J. Vet. Res. **12:**194–198.