Growth of *Listeria monocytogenes* on Vacuum-packaged Beef

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**ABSTRACT**

Pieces of beef striploin (400 g) were inoculated with *Listeria monocytogenes* strain Murray B, vacuum packaged, and stored at either 0°C or 5.3°C. Growth of the organism on the beef depended on the temperature of storage, the pH of the lean, and the type of tissue. Growth was more rapid at 5.3°C than at 0°C, and faster on strips of high pH (6.0-6.1) than on strips of low pH (5.5-5.7). During storage, the population of *L. monocytogenes* was higher on fatty tissue than on lean principally because growth occurred earlier on the fat. When low pH strips were held at 5.3°C, listeria grew from an initial count of 2.5 x 10^2 CFU/cm^2 to 3 x 10^7 CFU/cm^2 in 16 d on the fat, and in 20 d, to 10^6 CFU/cm^2 on the lean and to 5 x 10^7 CFU/ml in the purge fluid. After storage at 0°C for 76 d, the populations reached were 10^6 CFU/cm^2 on the fat, 10^4 CFU/cm^2 on the lean, and 3 x 10^5 CFU/ml in the purge fluid. When high pH strips were held at 0°C for 10 weeks, listeria grew from an initial population of 1.5 x 10^3 CFU/cm^2 to just over 10^7 CFU/cm^2 on the fat, 2 x 10^5 CFU/cm^2 on the lean, and 4 x 10^6 CFU/ml in purge fluid.

Recent foodborne outbreaks of listeriosis have raised concerns about the presence of *Listeria monocytogenes* in foods. *L. monocytogenes* is known to occur on raw meats (2,12,13). Growth on such meats during refrigerated storage could increase the risk of numbers of cells surviving a mild cooking process. It could also increase the risk of cross-contamination of cooked foods.

While published evidence appears conflicting, it suggests that little growth of *L. monocytogenes* occurs on chilled, raw meat. Although Khan et al. (8) obtained growth on sterile lamb at 8°C, Gouet and co-workers (5) found that *L. monocytogenes* did not grow on sterile beef mince during 17 d storage at this temperature. Gouet et al. (5) only observed growth of listeria at 8°C after the meat was spoiled by pseudomonads. They suggested that the resulting high pH (>6.2) and/or degradation products of meat were needed for growth. Variable results were obtained when the ability of *L. monocytogenes* to grow at 4°C in sterile muscle fluid was examined (8,9). Buchanan et al. (1) observed that there was no growth of *L. monocytogenes* in hamburger stored at 4°C for 7 d, but that numbers increased about 10-fold in irradiated (42 kGy) hamburger. Johnson et al. (7) also found that there was no detectable growth of listeria in packaged beef mince held at 4°C for 14 d. At

0°C, no significant growth occurred on sterile lamb meat held for 24 d (8).

The aims of the experiments reported here were to test (a) the ability of *L. monocytogenes* strain Murray B to grow on vacuum-packaged beef stored at either 0 or 5°C, (b) the influence of the pH of the lean on growth, and (c) whether growth was different on fatty and lean tissue.

**MATERIALS AND METHODS**

**Inoculum**

A 0.1 ml volume of stock culture of *L. monocytogenes* strain Murray B, previously grown at 37°C, was added to 10 ml tryptose soya broth (Oxoid, Basingstoke, UK) supplemented with 0.3% yeast extract (Oxoid). This culture was incubated at 10°C for 3 d and then 0.06 to 0.25 ml (about 80 Klett units; Klett-Summerson colorimeter with No. 66 filter) diluted in 1 L of distilled water to provide the inoculum for the meat.

**Meat packaging**

Beef striploins (*M. longissimus dorsi*) with the overlying fat intact were obtained from a commercial boning room, and each striploin was cut into 7 to 8 portions, each portion weighing about 400 g. Meat was selected after measuring the 

pH (range 5.0 to 5.5°). Two separate experiments were done on samples from each treatment.

**Microbiological sampling**

Fluid that exuded from the packaged beef during storage (purge fluid) was diluted in 0.1% peptone. Using a cork borer, five samples (each 5 cm² x ca. 0.4 cm deep) were taken from the fatty tissue and five from the lean surface. The separate lean and fatty tissue samples were blended for 1 min with 90 ml 0.1% peptone water in a Colworth Stomacher, Model 400. Portions (0.1 ml) of appropriate dilutions in 0.1% peptone were surface plated on tryptone soya agar (Oxoid) supplemented with 0.2%
yeast extract (Oxoid) and 0.2% glucose (TYSG) and on the selective agar of Lee and McClain (10) modified by the addition of 0.1% mannitol, 0.05% esculin, and 0.05% ferric citrate (ELPM). Plates were incubated at 37°C for 24 h and suspected listeria colonies counted. On TYSG, colonies of listeria were bluish in color when viewed with 45° incident light; on ELPM, colonies of listeria were surrounded by a black halo. Three to five representative colonies were purified and isolates confirmed as being L. monocytogenes by the following tests: gram reaction, hemolysis of sheep red blood cells, tumbling and umbrella motility, oxidase, catalase, methyl red, Voges-Proskauer, nitrate reduction, and fermentation of glucose, rhamnose, esculin, mannitol, and xylose.

To estimate the numbers of other bacteria growing on the packaged beef, plates of TYSG were also incubated at 5°C and colonies counted after 7 and 14 d, and at 25°C colonies were counted after 2, 3, and 4 d. Three to five colonies of each colonial type were isolated onto TYSG and identified either as listeria or as other psychrotrophic organisms (gram reaction, morphology, motility, catalase, oxidase, oxidative-fermentative utilization of glucose, and growth on streptomycin thallous acetate actidione agar; 6).

The log₁₀ counts of colony forming units (CFU) for each time point in the duplicate experiments were averaged and the mean values plotted.

**pH measurements**

At each sampling time, 5 to 6 g of lean tissue was blended with nine times its mass of distilled water and the pH measured (Radiometer TTT2). Purge fluid was warmed to room temperature and its pH measured.

**RESULTS**

**Growth at 5.3°C - lean pH 5.5-5.6**

The mean pH of the lean tissue in these experiments was pH 5.54 (range 5.50-5.60), while the mean pH of the purge fluid was pH 5.42 (range 5.40-5.50). Fig. 1 (a) shows the changes in the number of CFUs of L. monocytogenes on vacuum-packaged beef during storage at 5.3°C. There appeared to be little if any lag period before listeria began growing on the fatty tissue at a generation time of about 1.25 d. L. monocytogenes grew to reach 3 x 10⁷ CFU/cm² after 16 d storage. After 20 d, listeria reached a population of 5 x 10⁷ CFU/ml in the purge fluid and 10⁸ CFU/cm² on the lean. Growth on lean tissue was preceded by a lag period of 5 to 6 d.

At the time of packaging, psychrotrophs other than listeria were not detected on the meat (<25 CFU/cm²). However, during storage a psychrotropic flora appeared which grew more rapidly than listeria on lean and fatty tissue and in purge fluid (Fig. 1b). Lactic-acid bacteria and Enterobacteriaceae were the major components of this psychrotrophic flora. In the latter stage of storage, the numbers of these psychrotrophs exceeded the numbers of listeria both on lean tissue and in purge fluid. However, on fatty tissue, listeria outnumbered other psychrotrophs throughout the 30-d storage period.

**Growth at 0°C - lean pH 5.5-5.7**

The mean pH of the lean tissue used in these experiments was pH 5.58 (range 5.50-5.70), and the mean pH of the purge fluid was pH 5.47 (range 5.43-5.62).

Growth of L. monocytogenes on vacuum-packaged beef of this pH at 0°C was very slow (Fig. 2a). The fastest growth occurred on the fatty tissue where the generation time was about 7.5 d. Growth on lean tissue was severely inhibited. No growth appeared to take place until after 61 d storage. After storage for 76 d, the mean number of CFU of listeria on the lean was only about five times the initial count. The packaged beef sampled at 76 d had pH values of 5.64 and 5.70.

During storage, the major psychrotrophs other than listeria that grew on the packaged beef were lactic-acid bacteria. These psychrotrophs grew faster than listeria (Fig. 2b). In spite of the very low initial count of psychrotrophs...
(lactic-acid bacteria were not detected on fatty and lean tissue until 3 weeks storage), lactic-acid bacteria outnumbered listeria on lean tissue, and in purge fluid after the meat had been held for 34 d.

**Growth at 0°C - lean pH 6.0-6.1**

The mean pH of the lean tissue in the vacuum-packs was pH 6.03 (range 6.00-6.10), and the mean pH of purge fluid was pH 5.85 (range 5.80-5.90).

While there was a lag period of about 3 weeks before *L. monocytogenes* began to grow on lean tissue and in purge fluid, there appeared to be little if any lag on fatty tissue (Fig. 3a). On fatty tissue, the generation time of listeria was about 5.5 d. After 10 weeks storage, the count of *L. monocytogenes* increased by 500-fold on the lean and by about 9,000-fold on the fat. Growth of *L. monocytogenes* on lean and fat and in purge fluid was faster in packs of beef of pH 6.0-6.1 than in packs where the pH was 5.5-5.7.

The growth of psychrotrophs other than listeria is shown in Fig. 3(b). The principal flora growing on fatty tissue were lactid-acid bacteria and *Brochothrix thermosphacta*. On lean and in the purge fluid, lactic-acid bacteria were the dominant organism with *B. thermosphacta* forming 10% or less of the microflora. On all three sites, these psychrotrophs grew faster than *L. monocytogenes*, and at most sampling times outnumbered listeria.

**DISCUSSION**

The growth of *L. monocytogenes* on vacuum-packaged beef was affected by the temperature of storage, the type of tissue, and the pH of the lean.

When striploins whose lean pH was 5.5-5.6 were held at 5.3°C, the growth of listeria was considerably faster on lean and fat and in the purge fluid than when striploins of similar pH were held at 0°C. The populations of listeria reached in 2 weeks storage at 5.3°C were greater than those reached in 11 weeks storage at 0°C.

Similarly, growth of listeria was considerably more extensive on lean and fat and in purge fluid from striploins of pH 6.0-6.1 than in these tissues from striploins of pH 5.5-5.7. At 0°C, the minimum pH for growth of *L. monocytogenes* strain Murray B on the lean of vacuum-packed beef appears to be close to 5.5-5.6. At 5.3°C, the organism is able to grow on lean at this pH. While the minimum pH for growth at 4°C is in the range pH 5.23-5.45 for laboratory media adjusted with hydrochloric acid, the minimum pH may be somewhat higher when lactic acid is the acidulant (3). In postrior muscle, lactic acid is the major acidulant. The importance of pH in controlling growth of *L. monocytogenes* is apparent in the increase in numbers of the organism that occurs during ripening of Camembert cheese when the pH rises to about pH 6 (11). Product pH has also been suggested as one of the factors regulating growth of listeria on cooked, processed meats (4).

In all storage experiments, whether the temperature was 0 or 5.3°C or whether the pH of the lean was 6.0-6.1 or 5.5-5.7, listeria maintained higher populations on fat than on lean. Mostly this seemed to be the consequence of a shorter lag phase on fat than on lean. The shorter lag on fat may be a consequence of its pH being near neutrality. More oxygen may also be available at the fat surface since lean has a greater reducing ability than fat.

The numbers of listeria/ml of purge fluid were at least 10 times the count/cm² of lean. It is difficult to estimate the lag period for growth in this fluid since the first sample was not obtained until after 5-7 d storage, and listeria in the fluid would include cells washed from the lean surface. However, it appears from the experiments with striploins of pH 5.5-5.7 stored at 0°C (Fig. 2) that growth in the purge fluid could precede that on lean.

In the three treatments, the normal psychrotrophic flora associated with vacuum-packaged beef grew at a faster rate than did listeria. However, listeria were able to grow even when significantly outnumbered by this flora. For instance, after 4 weeks storage, listeria grew in purge fluid in the presence of more than 10⁷ other bacteria/ml (Fig. 3).

The data in Fig. 1 (a) and 1 (b) indicate that there were considerable lag periods before listeria began growing on lean of pH 5.5-5.7 at either 5.3 or 0°C. It is therefore not surprising that no significant growth occurred in minced beef of initial pH ca. 5.6 held for 14 d at 4°C (7). Differences in the pH of hamburger samples used by Buchanan et al. (1) may explain why growth was observed with irradiated, but not normal, hamburger stored at 4°C for 7 d. Similarly, some of the variability in growth responses of *L. monocytogenes* in sterile muscle-fluid samples obtained by Khan et al. (8,9) may have been due to differences in pH. In published works, the inocula used on meat have been grown at 30 to 37°C. For the work reported here, the inocula were grown at 10°C. It is possible that lag periods on chilled meat may be longer for inocula grown at the higher temperatures.

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**Figure 3. Changes in the number of microorganisms on fat (Δ), lean (O), and in purge fluid (Q) of vacuum-packaged beef of pH 6.0-6.1 stored at 0°C: (a) growth of *L. monocytogenes*, (b) growth of psychrophils other than listeria.**

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REFERENCES


