Incidence and Recovery of Listeria from Chicken with a Pre-enrichment Technique

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ABSTRACT

Eighty frozen chickens from four processors were purchased from retail stores in Brisbane. Forty-eight fresh chicken carcasses and 32 (16 hot and 16 chilled) wash-water samples from each of the four processors were also collected. The isolation of Listeria was achieved by a pre-enrichment procedure which allowed the recovery of injured cells. Listeria monocytogenes was isolated from 12 (15%) of the frozen chickens. Nine (11.2%) isolates were confirmed to be serotype 1 and three (3.8%) serotype 4. Fourteen (17.5%) of the frozen chickens were also contaminated by Listeria innocua. One (2.1%) sample of the fresh chickens yielded L. monocytogenes serotype 1 and five (10.4%) had L. innocua. L. monocytogenes serotype 1 was recovered from two (6.2%) samples of chilled wash water, but no Listeria were detected in hot wash water.

Outbreaks of listeriosis due to consumption of contaminated dairy products (6), coleslaw (13), and other foods (7) have resulted in a number of studies to establish the incidence of Listeria in the food supply (3, 14). As a result of the various investigations, Listeria have been isolated from at least 40 species of animals (5), including chicken (2, 7). Although many of the foods examined have not been implicated in listeriosis, they are potential sources of Listeria infections because Listeria is zoonotic, widespread (14), and psychrotrophic (11), and its infectious dose for humans may be relatively low, 10^2 to 10^5 cells (6), particularly in immunocompromised people.

As Listeria may also occur in small numbers in food (9), pre-enrichment in suitable broth media is necessary to recover the organism. Most of the proposed methods for Listeria isolation use a direct selective enrichment procedure (8, 9). However, because foods are subjected to many different treatments, microorganisms can be present in different physiological stages and with varying degrees of sublethal injury (15). Those Listeria cells that have sustained damage from the various processes may be susceptible to suppression by the inhibitors in the selective enrichment broth, and the recovery of Listeria from these liquid media without resuscitation may not be successful.

Tryptone soya yeast extract broth (TSYE broth) has been found to be a suitable growth medium for Listeria (10). In the course of previous studies with 70 samples of dairy products, it was observed that during pre-enrichment in TSYE broth, pH decreased to between 5.5 and 6.0 which may suppress the resuscitation of impaired Listeria (12). For this reason, it was considered necessary to buffer this medium even for use with non-dairy food products. It was also found desirable to incubate the broth at 7°C in order to suppress the growth of competing bacteria which may have faster growth rates at mesophilic temperatures.

The objectives of this report were to determine an optimal method for the recovery of Listeria from food and the incidence of Listeria in chickens processed in Brisbane, Australia.

MATERIALS AND METHODS

Samples

Because chicken abattoirs in Queensland operate under different processing conditions, two large and two small processors were selected. The large processors use automated plants, while the small processors operate manual plants.

Five raw frozen chickens from each of the four processors were purchased from retail shops in Brisbane, and the survey was repeated on four successive weeks, so that a total of 20 frozen chickens from each of the four processors were analyzed.

Twelve raw fresh chickens from each of the four processors were also collected. Three birds were obtained after each of the four major stages of processing, i.e. hot wash immersion, defeathering, evisceration, and chilled wash immersion, on the processing day.

Four samples of hot wash water and four samples of chilled wash water from each of the four processors were also sampled at different times over a period of about 6 h.

The samples were transported to the laboratory in insulated boxes and tested on the day of sampling. Chickens were placed into sterile plastic bags (Stomacher, Seward 3500, London, UK) and weighed to the nearest 1 g. The frozen chickens were thawed for 4 to 5 h in a 37°C incubator prior to testing.

Pre-enrichment broth (g/L)

Tryptic soy broth (Difco, Detroit, MI), 30.0; yeast extract (Difco), 6.0; K_2HPO_4 (BDH), 3.5; and NaH_2PO_4.H_2O (Merck), 1.7 were dissolved in distilled water, and the pH was adjusted to 7.5.
with solid NaOH so that after autoclaving at 121°C/20 min, the pH of the broth was 7.3±0.1. The broth was dispensed in 225 ml
volumes in 500-ml screw capped bottles prior to sterilization.

**Selective enrichment broth**

UVM modified Listeria enrichment broth (Difco) was prepared according to Frazer and Sperber (4) and dispensed in 10 ml
quantities in 28-ml screw capped bottles prior to sterilization.

**Diagnostic agar**

McBride Listeria agar (Difco) and Oxford agar (Oxoid) were prepared and used as directed by the manufacturers.

**Agar slants (g/L)**

Polypeptone (BBL), 10.0; yeast extract (Difco), 5.0; NaCl (May and Baker), 5.0; and agar (Difco), 15.0 were dissolved in
distilled water and the pH was adjusted to 7.2±0.1. The agar was
dispensed in 12 ml volumes in 28-ml bottles with screw caps and
autoclaved at 121°C/15 min.

**Reference organism**

Listeria monocytogenes UQM 3172 was obtained from the
University of Queensland, Brisbane, and used as the reference
organism. The culture was put through all the stages of the testing
procedure on each occasion that samples were tested.

**Isolation procedure**

Five hundred milliliters 0.1% peptone solution, pH 7.0, were
added to the stomacher bags and the chickens rinsed and mas-
saged for 2 min (1). A volume of liquid equivalent to that which
would cover 100 cm$^2$ of surface area of chicken (1) was inocu-
lated into 225 ml pre-enrichment broth and incubated at 7°C for
7 d. With the wash water samples, 25 ml inocula were used. One
milliliter of the pre-enrichment broth was transferred into selec-
tive enrichment broth and incubated at 37°C for 48 h.

Those broth cultures that changed colour from straw to black
were streaked both undiluted and diluted 1:10 in 0.5% KOH
solution for 20 sec on each half of the same plate of diagnostic
agar media. The inoculated plates were incubated at 37°C for 48
h. McBride agar plates were viewed by the oblique transillumina-
tion technique (8,9) and Oxford agar plates were examined with-
out magnification.

Typical Listeria colonies were harvested and inoculated into
10 ml pre-enrichment broth and onto agar slants. The liquid culture
media were incubated at 22°C for 18-24 h, while the solid culture
media were incubated at 37°C for 18-24 h. Oxidase (Marion
Scientific), Gram stain and haemolysis (8) tests were performed
from the agar media. Blood agar plates were inoculated by punc-
tiform inoculation and incubated at 37°C for 48 h. Catalase rea-
tion and tumbling or spinning mobility were determined from the
liquid cultures. Isolates were confirmed as Listeria biochemically
(Minitek Gram Positive Set, BBL) and serologically (Difco List-
eria antisera). Agglutination tests were carried out by the macro-
scopic tube technique according to manufacturer’s instructions
after harvesting the cells from nutrient agar.

**Comparison of methods**

The developed method was compared to the U.S. Food and
Drug Administration (FDA) procedure (8) for 36 samples of dairy
product and to the Association of Official Analytical Chemists
(AOAC) procedure (9) for 13 samples of meat. This method was
also evaluated against a proprietary ELISA kit (TECRA, Bioen-
terprises, Sydney, Aust.) which stipulated the use of a direct se-
lective enrichment step as part of the testing procedure in which
86 samples were examined which included 60 environmental, 2
ice-cream, 5 cheese, 16 meat, 3 salami, and 2 reference organism
samples.

**RESULTS AND DISCUSSION**

No Listeria were found in dairy factory environmental
samples or cheese samples by either the FDA method or the
developed method. From mildly cooked meat cooked in a
microwave oven, five Listeria isolates were detected by the
AOAC method and eight by the developed method, i.e.,
five from the same samples plus in an additional three
drugs.

Listeria was isolated by both the developed method
and the ELISA kit from one ice-cream and one salami; both
methods correctly identified the reference organism. However,
the TECRA kit isolated Listeria from six meat samples
and the developed method recovered Listeria from eight
samples, i.e., six from the same samples plus in an addi-
tional two samples. Similarly, the developed method detected
L. monocytogenes in a salami and in a pate, while the
TECRA kit gave negative results for Listeria in these
samples.

The developed method thus gave greater recoveries of
Listeria from meat samples than either the AOAC method
or the TECRA kit.

The occurrence of Listeria on frozen chicken is pre-
sented in Table 1. Overall, 26 (32.5%) of 80 samples were
contaminated by Listeria. Listeria monocytogenes serotype
1 was recovered from nine (11.3%) and serotype 4 from
three (3.8%) carcasses. Listeria innocua was found on 14
(17.5%) birds.

<table>
<thead>
<tr>
<th>TABLE 1. Incidence of Listeria in frozen chicken.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processor</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Average</td>
</tr>
</tbody>
</table>

However, when freshly processed chickens were exam-
ined, the frequency of contamination by Listeria was con-
siderably lower as evidenced in Table 2. L. monocytogenes
serotype 1 was isolated from one (2.1%) and L. innocua
from five (10.4%) chickens.

The wide variation in the detection of Listeria between
frozen and fresh chickens cannot be explained. However,
Listeria are resistant to freezing, and freezing and thawing
procedure may have reduced the number of competing
microorganisms, allowing Listeria to be isolated more read-
ily.
**TABLE 2. Incidence of Listeria in fresh chicken during processing.**

<table>
<thead>
<tr>
<th>Processor</th>
<th>No. tested</th>
<th>Process</th>
<th>L. monocytogenes</th>
<th>L. innocua</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>Post hot wash</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Post hot wash</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Post hot wash</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Post hot wash</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>Post defeathering</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Post defeathering</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Post defeathering</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Post defeathering</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>Post evisceration</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Post evisceration</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Post evisceration</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Post evisceration</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>Post chilled wash</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Post chilled wash</td>
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<td>3</td>
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<td>1 Type 1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Post chilled wash</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Average**

|          | 1 (2.1) | 5 (10.4) |

The temperature in the hot water tanks ranged from 56 to 67°C and in the chilled water tanks, the temperature varied between 0 and 9°C. The temperature was measured at hourly intervals over a period of about 6 h. As documented in Table 3, no Listeria were recovered from the hot water, but two L. monocytogenes isolates were found in the processing plant where the temperature of the chilled water tank was 9°C.

Thirty-four Listeria were recovered by Oxford agar and 32 cultures were isolated by McBride agar. The two additional L. monocytogenes from the Oxford agar were from the same source, one isolate being serotype 1 and the other serotype 4. A total of 19 L. innocua were found. Six (31.6%) of these cultures cross reacted with the antiserum to serotype 4 and they originated from the same processor.

In Tables 1, 2, and 3, processors 1 and 2 refer to large automated plants which had various quality control systems in operation. These large processors had a capacity to produce 40,000 to 63,000 frozen chickens per day. Listeria were present in raw frozen chicken from both processors. Processors 3 and 4 were small, manual plants which produced 300 to 500 frozen chickens per day. Processor 3 had the highest frequency of contamination by Listeria in chickens.

Table 1 shows 32.5% of frozen chickens were contaminated by Listeria, i.e., 15% by L. monocytogenes and 17.5% by L. innocua. These results are comparable to those of Bailey, Fletcher, and Cox (2) who found that in the United States, 38% of fresh chickens were contaminated by Listeria, with L. monocytogenes being present in 23% of the samples. However, Wales, Kwantes, and Isaacs (7) reported the incidence of contamination of broilers by L. monocytogenes was 53%. Although the incidence of L. monocytogenes in frozen chickens in Brisbane was relatively low compared to reports from some parts of the world, it is essential to emphasize the importance of practicing proper hygiene and temperature control in hot and chilled water tanks in chicken processing plants. The temperature of the chilled water tanks should be less than 5°C, preferably 0-2°C. The chlorine level should be maintained at an appropriate level (e.g., 40 ppm of sodium hyperchloride). Sanitary operation, maintenance, and repair practices should also be appropriate to the equipment design to reduce the presence of Listeria in the plant environment which could contaminate the finished product if these procedures are not followed. This is necessary to minimize the incidence of Listeria in raw chicken in order to reduce the potential for listeriosis from improperly cooked chickens.

**ACKNOWLEDGMENTS**

Special appreciation is extended to Mrs. E. Jones and Miss D. Fairfull for their technical assistance. This project was supported by the Veterinary Public Health Branch, Queensland Department of Primary Industries.

**REFERENCES**


**TABLE 3. Incidence of Listeria in wash water.**

<table>
<thead>
<tr>
<th>Processor</th>
<th>Wash water temperature °C</th>
<th>Number tested</th>
<th>Number (%) positive Listeria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hot</td>
<td>Chilled</td>
<td>Hot</td>
</tr>
<tr>
<td>1</td>
<td>59 to 60</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>0 to 2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>60 to 62</td>
<td>3 to 9</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>62 to 67</td>
<td>0 to 7</td>
<td>4</td>
</tr>
</tbody>
</table>

**Average**

|          |              | 0             | 2 (0.2)                       |

*L. monocytogenes* type 4.
TABLE 3. pH determinations of shredded cabbage stored under various conditions.

<table>
<thead>
<tr>
<th>Day of storage</th>
<th>Modified atmosphere</th>
<th>Vacuum packaged</th>
<th>Aerobically packaged</th>
<th>Modified atmosphere</th>
<th>Vacuum packaged</th>
<th>Aerobically packaged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 ± 2°C</td>
<td>0-6°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.09±</td>
<td>5.10</td>
<td>6.10</td>
<td>6.61</td>
<td>6.75</td>
<td>6.42</td>
</tr>
<tr>
<td>2</td>
<td>5.06</td>
<td>5.32</td>
<td>5.94</td>
<td>6.76</td>
<td>6.56</td>
<td>6.65</td>
</tr>
<tr>
<td>3</td>
<td>4.10</td>
<td>4.10</td>
<td>4.26</td>
<td>6.69</td>
<td>6.55</td>
<td>6.13</td>
</tr>
<tr>
<td>4</td>
<td>3.70</td>
<td>4.64</td>
<td>4.16</td>
<td>6.71</td>
<td>6.51</td>
<td>6.48</td>
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<tr>
<td>7</td>
<td>3.81</td>
<td>3.99</td>
<td>4.07</td>
<td>6.64</td>
<td>6.54</td>
<td>6.61</td>
</tr>
</tbody>
</table>

*Initial pH values (day 0) were 6.81/6.78 (duplicate determinations).
*Data presented are means of duplicate determinations.

ACKNOWLEDGMENT

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