Incidence of *Listeria* spp. in Pre-cooked, Chilled Chicken Products as Determined by Culture and Enzyme-linked Immunoassay (ELISA)

KEVIN G. KERR*, NATHANIEL A ROTOWA, PETER M. HAWKEY, and RICHARD W. LACEY

Department of Microbiology, University of Leeds, Leeds, West Yorkshire LS2 9JT, United Kingdom

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**ABSTRACT**

One hundred and two samples of ready-to-eat, pre-cooked, chilled chicken were examined for *Listeria* spp. using culture and a commercially available enzyme linked immunoassay (ELISA). Overall, 29 (28%) samples contained *Listeria* spp. as determined by culture, *Listeria monocytogenes* being present in 27 (26.5%) samples. In comparison with culture, the ELISA yielded no false positives. However, the technique detected *Listeria* spp. in only 24 of 29 culture-positive samples.

Human listeriosis is caused by the gram-positive bacillus *Listeria monocytogenes*. In adults, infection with this organism usually manifests as meningitis often in association with septicaemia. In the pregnant woman, *L. monocytogenes* may cause a self-limiting “flu-like” illness often resulting in abortion, stillbirth, or the premature birth of a live child with neonatal listeriosis. The overall case fatality ratio for human listeriosis is approximately 30% (16). Individuals at extremes of age and those with impaired immunity have been identified as being particularly at risk of developing listeriosis; however, the disease can affect previously healthy individuals (1).

There is now considerable microbiological and epidemiological evidence from both sporadic and epidemic cases of human listeriosis to suggest that contaminated foodstuffs are the primary means of transmission of *L. monocytogenes*. Various foodstuffs have been associated with outbreaks and sporadic cases of listeriosis; these include soft cheese, milk, coleslaw, and turkey franks (1,2,5,13,18). Several reports have identified cooked and undercooked chicken as a source of listeriosis (9,11,19). In order to investigate this problem further, we examined samples of pre-cooked, chilled chicken using both conventional cultural and ELISA techniques to determine the prevalence of *L. monocytogenes* in these products.

**MATERIALS AND METHODS**

One hundred and two pre-cooked, chilled chickens were purchased from retail outlets. All samples were labelled ready-to-eat. Products examined included whole chickens and chicken portions, e.g., half, quarter chickens, and chicken breasts. All products were within their “sell-by” dates and were processed within 2 h of purchase.

Twenty-five gram portions of each chicken were homogenized in 225 ml of *Listeria Enrichment Broth* (LEB) (Oxoid, Basingstoke, UK) containing 12 mg/l acriflavine, 40 mg/l nalidixic acid, and 50 mg/l cycloheximide in a stomacher (Denley Tech, Billingshurst, West Sussex, UK) for 2 min. A 200 μl portion of homogenate was plated onto Oxford agar (3, Oxoid, Basingstoke, UK), and the remainder incubated at 30°C in air for 24 h and then subcultured to Oxford agar. In addition, 1 ml of broth was transferred to 9 ml of LEB and incubated for a further 24 h, after which a final subculture to Oxford agar was made and the ELISA procedure was performed. The Tekra listeria immunoassay (Bioenterprises Pty., Roseville, Australia) was used according to the manufacturer’s instructions. ELISA results were determined visually.

For identification of organisms, eight black (esculin hydrolysing) colonies from each Oxford agar plate were subcultured to horse blood agar for purity and observation of hemolysis. Isolates of *L. monocytogenes* were serotyped by slide agglutination using commercially available types 1 and 4 antisera (Difco, Detroit, MI).
RESULTS

As determined by culture, 29 of 102 samples (28%) contained Listeria spp.; of these, 20 (69%) yielded L. monocytogenes and a further 7 (24%) L. monocytogenes and L. seeligeri in combination. Serotypes of the L. monocytogenes isolates are shown in Table 1. Listeria seeligeri alone was found in one sample (3%) as was L. innocua. Thus, L. monocytogenes was found in 26.5% of all samples tested. Confirmed L. monocytogenes isolates were only obtained when enrichment was used. In comparison with cultural methods, the ELISA yielded no false positives. However, of 29 samples positive by culture only 24 were positive by ELISA. With two of the culture positive ELISA negative enrichment broths, quantitative counts were determined prior to the ELISA procedure. The numbers of Listeria in these enrichment broths were 2 x 10^3 and 1.5 x 10^3 organisms/ml. Reincubation of these broths for a further 24 h, at which time levels were >10^9 organisms/ml, gave positive results in the ELISA kit on retesting.

TABLE 1. Incidence of Listeria spp. in 102 cook-chill chicken products.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples positive</th>
<th>Serotype of L. monocytogenes^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. monocytogenes</td>
<td>20</td>
<td>1 (16) 4 (4)</td>
</tr>
<tr>
<td>L. monocytogenes &amp;</td>
<td>7</td>
<td>1 (4) 4 (2)</td>
</tr>
<tr>
<td>L. seeligeri</td>
<td></td>
<td>1 and 4 1 (1)</td>
</tr>
<tr>
<td>L. innocua</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. seeligeri</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>29</strong></td>
<td></td>
</tr>
</tbody>
</table>

^aNumber of strains in parentheses.

DISCUSSION

This study has demonstrated that using cultural methods 28% of retail pre-cooked, chilled chicken products are contaminated with Listeria spp. In 94% of cases, this contamination is with L. monocytogenes either alone or in combination with other species.

The isolation of Listeria spp. by conventional culture, however, is hampered by a lack of a standardized method and by the labor intensive nature of these techniques. These problems have led to the introduction of new methodologies such as DNA hybridization and ELISA (12,15). In this study a commercially available ELISA kit was 100% specific and 82.8% sensitive compared with conventional culture. Our results suggest that the ELISA may yield false negatives in samples with very low numbers of Listeria spp. and that a longer incubation period may be required when using the ELISA with foods in which very low levels of listeria might be expected. Nevertheless, our results compare very favorably with those of Heisick et al. (8) who examined 71 samples of milk and 238 samples of fresh vegetables using four methodologies including an other commercially available ELISA kit (Organon, Listeria Tek). This ELISA detected all 59 positive milk samples but yielded 5 false positives. The kit also detected 30 or 44 positive vegetables samples with 17 false positives (8).

The origin of Listeria spp. in cooked chicken is uncertain. Listeria monocytogenes is known to be present in raw poultry whether fresh or frozen. Between 14.7 and 60% of samples have been found to contain L. monocytogenes (6,17) and the organisms may survive heat processing. The current UK Department of Health guidelines recommend that pre-cooked, chilled food items should be processed so that “the temperature throughout the food should be held above 70°C for not less than 2 minutes” (4). However, these guidelines apply only to institutional cook-chill production units and not to commercial facilities. Alternatively, the presence of these organisms in such products may represent post-heating contamination. Harrison and Carpenter have recently demonstrated the survival of, albeit large, populations of L. monocytogenes in chicken breasts using moist heat (7). The same authors express concern that survivors of marginally processed products could grow to high levels during extended periods of refrigeration. It is of particular concern that we have found these products, in many retail outlets, to be refrigerated under sub-optimal conditions (10).

It is interesting to note that L. innocua was identified in only 1 of 102 (0.9%) samples examined. Skovgaard and Morgen found this organism in 16 of 17 (94%) samples of raw chicken (20). We detected L. innocua in all of seven samples of raw chicken examined for Listeria spp. (Kerr KG, Rotowa NA unpublished observations). If it is postulated that L. monocytogenes in cooked poultry is due to survival of the heating process, then the low prevalence of L. innocua in such products may mean that these organisms are considerably less thermotolerant than L. monocytogenes. Currently, we are investigating this hypothesis.

All products examined in this study were labelled “ready to eat” and the presence of L. monocytogenes in these products is of great concern. In all samples tested the organism was detected on enrichment only. Yet, in a case of materno-fetal listeriosis, L. monocytogenes obtained from the presumed food source, pre-cooked chicken, was isolated on enrichment only (11). In addition, products we examined were well within their “sell-by” dates, and low levels may have increased following extended refrigeration by both the retailer and consumer. Furthermore, discussions regarding the significance of low numbers of L. monocytogenes in foodstuffs often fail to take into account interstrain differences in virulence and also host variables such as functional or anatomical achlorhydria.

REFERENCES

Sinha, con't. from p. 587

effective in stabilizing the lactose-fermenting trait of lactococci.

The acceleration of ripening and flavor development in Cheddar cheese is known to result from the use of Lac mutants of lactococci as an adjunct to the normal starters (1,6). An understanding of the nature and mechanisms of the appearance of Lac mutants under different physiological conditions would enable development of suitable strains for use in accelerating cheese ripening. The appearance of Lac cells at a high frequency in the present investigation suggests that it would be possible to utilize this simple technique for isolating Lac variants. Such technique could also be useful in isolating additional mutants deficient in other plasmid-linked genetic traits in lactococci.

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