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Occurrence of *Listeria* species in ready to eat foods

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SUMMARY

Over 8000 ready to eat foods were examined for the presence of *Listeria* species. Overall, 5% of foods were found to contain these organisms. Higher occurrence was found in some foods such as chicken (11%) and fish (14%). Most of the *Listeria* species isolated were *L. monocytogenes* (49%) and *L. innocua* (36%) with lower numbers of other species. No seasonal pattern in the recovery of *L. monocytogenes* was found. Unsatisfactory or potentially hazardous levels of *L. monocytogenes* were found in 14 products (< 0·2%), mostly cooked meats. Undercooked chicken products appeared to present the greatest risk for the duration of this survey. The small number of samples which were potentially hazardous suggests that the risk to consumers is not high, and this is confirmed by the absence of clinical cases in the region during the period of study.

INTRODUCTION

Listeria have long been recognized as the cause of a range of serious human diseases [1]. Various surveys have shown them to be carried intestinally by 5–70% [2] and 0·5–92% of healthy humans [3], and by many animals [4, 5]. Exposure, consumption and carriage often do not lead to development of disease. Immunocompromised individuals including the immunosuppressed, elderly, pregnant, and those suffering a range of underlying diseases are primarily at risk. The spectrum of disease is broad, ranging from asymptomatic infection and carriage to uncommon cutaneous lesions, flu-like symptoms, various focal infections such as septic arthritis, conjunctivitis, urethritis, endocarditis [3], and the more serious and better recognised conditions of septicaemia, abortion and meningoencephalitis in humans [6]. The virulence [7–10] and health risks associated with different species and strains have been discussed [11], concluding that *Listeria monocytogenes* is the species of most concern, although the haemolytic species *L. seeligeri* and *L. ivanovii* are also recognised as potential pathogens [9, 12].

While *L. monocytogenes* has been found in silage [4], in faeces from both symptomatic and healthy individuals and animals, and at many environmental sites [5, 13], the more serious manifestations of infection and invasive disease are thought generally to be the result of eating contaminated food. Elucidation of the epidemiology is nevertheless incomplete [8]. Careful food production, cooking and

handling are required to minimize bacterial growth and exposure to the organism [6, 14, 13]. Outbreaks, superimposed on rare, sporadic cases of disease [8], have drawn attention to the presence of *L. monocytogenes* in certain foods such as undercooked chicken, cook-chill meals, cheese and pâté [14]. However, most sporadic cases cannot be linked to a specific food [3].

The infectious dose for humans is unknown and may vary widely depending on several factors, of which the immune status of the individual and the virulence of the strain ingested are probably the most important [3]. It has been suggested [15] that, for foods, $> 10^2$ colony forming units per gram (cfu/g) should be considered unsatisfactory and 10^3 cfu/g potentially hazardous.

The prevalence of *L. monocytogenes* in various foods has been shown in many studies to vary considerably [3, 6, 14, 16–20], making comparisons difficult. Nevertheless, chicken, meats, dairy products and seafood have sometimes been found to have a higher prevalence of the organism than other foods [3, 14, 16, 21, 22].

METHODS

Between January 1994 and January 1995, food samples were collected from retail displays and transported to the laboratory in cool boxes at < 4 °C. The majority of samples were cooked meats or other foods ready to eat without further processing and therefore considered to be of high risk to the consumer should pathogens be present. Samples also included ambient, chilled and frozen foods, dairy, plant and bakery products, prepared and restaurant meals, snacks, and a wide variety of other foods. Our findings for sandwiches will be published separately.

Foods were examined by enriching for *Listeria* spp. using the following method [23]. For all foods, 25 g was weighed into 225 ml Listeria Enrichment Broth (Oxoid CM862+SR144) and stomached for 60 s at normal speed (Colworth Stomacher 400, Seward and Co. Ltd). The broth was transferred to a honey jar and incubated at 30 °C for 24 h. After 24 and 48 h, the broth was streaked onto Oxford agar (Oxoid CM856+SR140) and incubated at 37 °C for 24 and 48 h.

Counts were performed by spreading 0·1 ml of the appropriate dilution on Oxford agar and incubating for 48 h at 37 °C.

Presumptive colonies (five) were subcultured on Columbia Agar (Oxoid) for 24 h at 37 °C and identified by catalase, motility, Gram stain, CAMP test, haemolysis and carbohydrate utilization (Rosco System, Lab M).

RESULTS

Table 1 shows the total numbers of foods examined in various categories and the numbers and percentages of those found to contain *Listeria* spp. The different numbers of samples examined in various categories means that percentages cannot be compared directly. The number and percentage of isolates which were *L. monocytogenes* are not shown because the low numbers of samples examined in some categories could make these figures misleading. Typically, *L. monocytogenes*

Table 1. *Foods containing* Listeria *spp.*

Food	Number examined	Number containing *Listeria* spp.	% containing *Listeria* spp.
Meat products			
Bacon	20	0	0
Beef	1295	44	3
Chicken	949	105	11
Lamb	37	1	3
Fish	49	7	14
Ham	1141	74	6
Pork	794	35	4
Fermented sausage	53	1	2
Pâté	222	2	1
Turkey	509	23	5
Mollusc/crustacean	74	8	11
Plant products			
Salads/vegetables	414	8	2
Rice	240	17	7
Dairy products			
Cheeses	49	2	4
Dairy ice cream	425	14	3
Non-dairy ice cream	511	18	4
Raw egg	29	1	4
Cooked egg	34	2	6
Cream	91	2	2
Cooked prepared meals			
Retail chill/frozen	488	13	3
Hospital chill/frozen	46	3	7
Soup	20	0	0
Bakery products			
Cream bun/cake	270	17	6
Other bakery products	30	8	27
Miscellaneous products	570	6	1
Total	8360	410	5

constituted around half of all listeria isolates, as shown in Table 2. Seafood, bakery and chicken products had the highest percentages of contamination.

Table 2 shows the numbers of *Listeria* spp. isolated from foods during the survey. Four hundred and ten listeria isolations were made on enrichment or at higher numbers from 8360 foods examined (5%). *L. monocytogenes* and *L. innocua* were by far the most commonly recovered species. Mixed cultures of these two species were recovered from six samples. Three isolates could not be speciated.

The *Listeria* species most commonly isolated in numbers greater than 10^2 cfu/g were *L. innocua* ($n = 15$) and *L. monocytogenes* ($n = 14$). Most of these unsatisfactory or potentially hazardous counts [15] were in cooked meat products. Levels of *L. innocua* exceeding 10^4 cfu/g were found in one sample of smooth liver garlic pâté and in a cream bun. Levels of $> 10^4$ cfu/g *L. monocytogenes* were found in two turkey products. *L. seeligeri* and *L. welshimeri* were found at levels between 10^2 and 10^4 cfu/g in two products each, and 200 cfu/g *L. murrayi* were found in one sample.

Table 2. *Isolation of* Listeria *spp.*

Species	*Number isolated** (%)
L. monocytogenes	199 (49)
L. innocua	146 (36)
L. seeligeri	38 (9)
L. welshimeri	20 (5)
L. spp.	3 (1)
L. murrayi	2 (0·5)
L. ivanovii	2 (0·5)
Total	410

* 8360 samples examined.

Table 3. Listeria *spp. in chicken products*

Chicken product	Number examined	Number containing *Listeria* spp.	% containing *Listeria* spp.
Chopped	152	26	17
Whole carcass	68	10	15
Joint	342	47	14
Burger	39	3	8
Spiced	42	3	7
Sliced	156	8	5
Pie	135	3	2
Other	15	0	0
Total	949	100	11

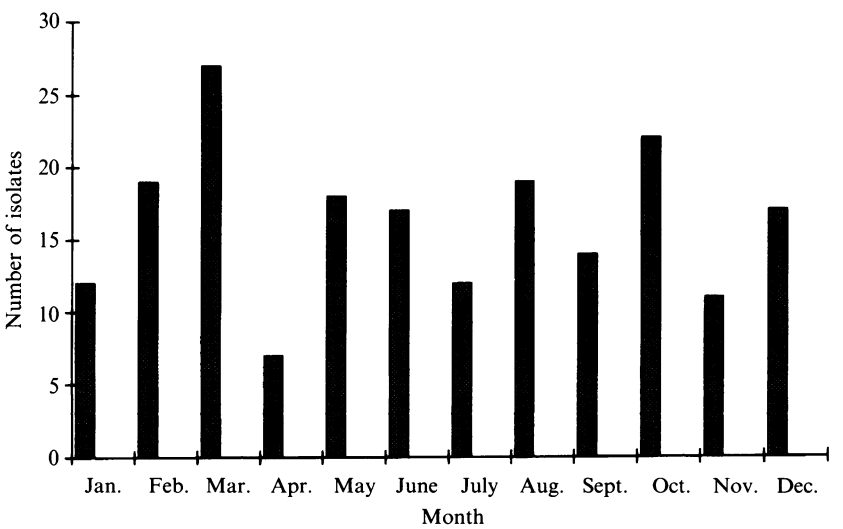

Fig. 1. Seasonal occurrence of *L. monocytogenes* in foods.

The number and percentage of chicken products containing *Listeria* spp. are shown in Table 3. Whole carcasses, joints, and chopped chicken were most frequently contaminated.

Figure 1 shows the monthly variation in numbers of *L. monocytogenes* isolations.

DISCUSSION

In apparent contrast to an earlier study [19] of 513 foods produced in Northern Ireland which showed 35% to contain *Listeria* spp. but none at > 100 cfu/g, the much larger number of samples reported here revealed a lower occurrence of the organisms but levels of listeria exceeding 100 cfu/g in 38 foods (0·5% of all foods; 9% of all foods containing listeria). Almost all of the foods sampled in the present survey were preselected as high risk foods and correspond to categories I and II of these workers (category I, foods intended for immediate consumption; category II, foods having received heating presumed sufficient to kill listeria but nevertheless intended to receive further cooking prior to consumption) so the samples may be considered broadly similar. In the earlier study a high proportion of prepared meals contained listeria [19], but in the present survey the numbers were much lower. The percentage of foods containing hazardous levels of *L. monocytogenes* (0·5%) was also lower than the 5% reported in a survey of 1159 foods [14] which included raw meats, unlike the present study.

This may be because samples in the present survey were sourced at retail from a large number of manufacturers, where the earlier study [19] reported on a smaller number at despatch from two processors only. This suggests differences between producers and processes, and generally reflects the risks in portioning, the mixing of microfloras from several foods, and low temperature storage which may select for listeria [8, 24]. The use of different isolation methods [19] may account for some differences between surveys.

The percentage of chicken products containing listeria and the large number of samples examined means that this is the food of greatest concern. This finding has been made by other workers [25]. It was found [22] that *L. monocytogenes* was common in raw poultry and that 8% of cooked poultry products ($n = 96$) contained *Listeria* spp. but none contained *L. monocytogenes*. These workers found *L. monocytogenes* to be consistently present on various food contact surfaces within a poultry production site. It is believed that most contamination during poultry processing occurs from food handlers, contaminated surfaces and equipment [13, 22, 26], but survival for several hours has been observed in aerosols [27] and may be of importance to cleaning regimes. The current study revealed a slightly higher rate of contamination (11%) than the 8% these workers [22] found in a smaller sample of cooked products, and a number of poultry products which contained in excess of 100 cfu/g listeria. In the present study, with an almost tenfold larger sample, 47 *L. monocytogenes* were isolated from cooked chicken products (5% of all chicken examined, 45% of all listeria, $n = 949$). Taking account of the larger sample size and increased power of the present survey, these results are not inconsistent with the earlier study [22].

Unlike these workers [22], however, I did not find that whole birds were significantly less contaminated than breast fillets, both having similar frequencies of contamination. This may be because the products sampled in the present survey came from a number of producers rather than the single factory studied in the earlier work. Sliced chicken had less than a third of the contamination rate of chopped chicken. This may reflect greater contamination from handlers than from slicing machines.

Pâté which has previously been recognized as a high risk food for listeria [28] was found to have a very low rate of contamination (0·5%), showing that effective controls appear generally to have been put in place. Nevertheless, a hazardous level of $> 10^4$ cfu/g *L. monocytogenes*, which warrants product recall, was detected in one pâté sample. Although a small sample size, only 1 of 36 soft cheeses was found to contain listeria, suggesting that the risk from such cheeses may have decreased.

The sporadic and non-seasonal nature of infections which has been noted by several investigators [6] was shown in the monthly isolation figures from the foods examined, reflecting the ubiquity of the organisms. These findings do not explain the annual rise in human cases and faecal carriage between July and September which has been noted [29, 21]. It has been suggested [21] that, unlike faecal carriage of *L. monocytogenes*, food borne contamination with this species is not seasonal. This theory is supported by our findings (Fig. 1) which showed considerable monthly variations in isolations which did not correlate with the rise in human listeriosis. However, no clinical cases were reported in Northern Ireland during the period of study.

As in other studies [17, 21, 30, 31], *L. innocua* and *L. monocytogenes* were found to be the most commonly isolated species. It has been observed [32] that the shorter generation time of *L. innocua* in enrichment broth may lead to underreporting of the slower growing *L. monocytogenes* in cultures where the two species are both present. Mixed cultures of these species were detected during the present study in two ham samples where the number of *L. innocua* was > 100 cfu/g and *L. monocytogenes* was detected only on enrichment. Similar situations may have existed in enriched samples where identifying five colonies was insufficient to detect both species. These workers [32] have commented that the detection of non-pathogenic *Listeria* species from foods does not preclude the presence of *L. monocytogenes* also. The numbers of samples containing *L. monocytogenes* were greater than those with *L. innocua* in the present study, while other workers have found these proportions reversed. This is likely to be due to the higher incubation temperature (37 °C) which was used in the present survey to select in favour of pathogenic *L. monocytogenes*. Incubation of Oxford agar at 30 °C as in most other studies is preferable for recovery of a wider range of less pathogenic *Listeria* species. This study found these two species to be seldom present in the same sample, but other workers have commented that the presence of other members of the *Listeria* genus indicates an increased risk of contamination with *L. monocytogenes* [33]. Given the similar ecological niches of *Listeria* species, the significance and utility of *L. innocua* as an indicator organism for pathogenic *L. monocytogenes* in subsequent samples perhaps warrants further investigation by time series experiments in the food production or retail environment.

These results emphasize the importance of thoroughly cooking chicken products before consumption, but do not sustain cause for concern with cheeses, or pâté, at least in the Northern Ireland region at this time. Workers in other countries have similarly reported low incidences of *Listeria* spp. in retail foods [25]. The small proportion of samples which were potentially hazardous suggests that risk to consumers is not high, and this is confirmed by the absence of clinical cases in the region during the period of study.

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REFERENCES

1. Gray ML, Killinger AH. *Listeria monocytogenes* and listeric infections. Microbiol Rev 1966; **30**: 309–82.
2. Gilbert RJ, Hall SM, Taylor AG. Listeriosis update. PHLS Microbiol Digest 1989; **6**: 33–7.
3. Farber JM, Peterkin JI. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev 1991; **55**: 476–511.
4. Gitter M. Veterinary aspects of listeriosis. PHLS Microbiol Digest 1989; **6**: 38–42.
5. Schuchat A, Swaminathan B, Broome CV. Epidemiology of human listeriosis. Clin Microbiol Rev 1991; **4**: 169–83.
6. Anonymous. *Listeria monocytogenes*. Recommendations by the National Advisory Committee on Microbiological Criteria for Foods. Int J Food Microbiol 1991; **14**: 185–246.
7. Portnoy DA, Chakraborty T, Goebel W, Cossart P. Virulence determinants of *Listeria monocytogenes* pathogenesis. Infect Immun 1992; **60**: 1263–7.
8. Rocourt J. *Listeria monocytogenes*: the state of the science. Dairy Food Environ Sanit 1994; **14**: 70–82.
9. Menudier A, Bosiraud C, Nicolas JA. Virulence of *Listeria monocytogenes* serovars and *Listeria* spp. in experimental infection of mice. J Food Protect 1991; **54**: 917–21.
10. Wernars K, Heuvelman K, Notermans S, Domann E, Leimeister-Wächter M, Chakrborty T. Suitability of the *prfA* gene, which encodes a regulator of virulence genes in *Listeria monocytogenes*, in the identification of pathogenic *Listeria* spp. Appl Environ Microbiol 1992; **58**: 765–8.
11. Hof H, Rocourt J. Is any strain of *Listeria monocytogenes* detected in food a health risk? Int J Food Microbiol 1992; **16**: 173–82.
12. Lessing MPA, Curtis GDW, Bowler ICJ. *Listeria ivanovii* infection. J Infect 1994; **29**: 230–1.
13. Marsden JL. Industry perspectives on *Listeria monocytogenes* in foods: raw meat and poultry. Dairy Food Environ Sanit 1994; **14**: 83–6.
14. Roberts D. *Listeria monocytogenes* and food: the U.K. approach. Dairy Food Environ Sanit 1994; **14**: 202–4.
15. Gilbert RJ. Provisional microbiological guidelines for some ready-to-eat foods sampled at point of sale; notes for PHLS examiners. PHLS Microbiol Digest 1992; **9**: 98–9.
16. Dillon RM, Patel TR. *Listeria* in seafoods: a review. J Food Protect 1992; **12**: 1009–15.
17. Adesiyun AA. Prevalence of *Listeria* spp., *Campylobacter* spp. *Salmonella* spp. *Yersinia* spp. and toxigenic *Escherichia coli* on meat and seafoods in Trinidad. Food Microbiol 1993; **10**: 395–403.
18. Harvey J, Gilmour A. Occurrence of *Listeria* species in raw milk and dairy products in Northern Ireland. J Appl Bacteriol 1992; **72**: 119–25.
19. Harvey J, Gilmour A. Occurrence and characteristics of *Listeria* in foods produced in Northern Ireland. Int J Food Microbiol 1993; **19**: 193–205.
20. Ribeiro CD, Burge SH. Developing microbiological guidelines for food: first results from cooked chicken portions. PHLS Microbiol Digest 1992; **9**: 100–2.
21. MacGowan AP, Bowker K, McLauchlin J, Bennet PM, Reeves DS. The occurrence and seasonal changes in the isolation of *Listeria* spp. in shop bought food stuffs, human faeces, sewage and soil from urban sources. Int J Food Microbiol 1994; **21**: 325–34.
22. Lawrence LM, Gilmour A. Incidence of *Listeria* spp. and *Listeria monocytogenes* in a poultry processing environment and in poultry products and their rapid confirmation by multiplex PCR. Appl Environ Microbiol 1994; **60**: 4600–4.
23. Kerr KG, Lacey RW. Isolation and identification of *Listeria monocytogenes*. J Clin Pathol 1991; **44**: 624–7.
24. Saguy J. Simulated growth of *Listeria monocytogenes* in refrigerated foods stored at variable temperatures. Food Technol 1992; **52**: 69–71.

25. Gohil VS, Ahmed MA, Davies R, Robinson RK. Incidence of *Listeria* spp. in retail foods in the United Arab Emirates. J Food Protect 1995; **58**: 101–4.
26. Hudson WR, Mead GC. *Listeria* contamination at a poultry processing plant. Lett Appl Microbiol 1989; **9**: 211–4.
27. Spurlock AT, Zottola EA. The survival of *Listeria monocytogenes* in aerosols. J Food Protect 1991; **54**: 910–2.
28. McLaughlin J, Hall SM, Velani SK, Gilbert RJ. Human listeriosis and pâté: possible association. B M J 1991; **303**: 773–5.
29. Newton L, Hall SM, McLauchlin J. Listeriosis surveillance. 1990. Comm Dis Rep 1991; **3**: R144–6.
30. Moore J, Madden RH. Detection and incidence of *Listeria* species in blended raw egg. J Food Protect 1993; **56**: 652–4, 660.
31. Varabioff Y. Incidence of Listeria in smallgoods. Lett Appl Microbiol 1992; **14**: 167–9.
32. Curiale MS, Lewus C. Detection of *Listeria monocytogenes* in samples containing *Listeria innocua*. J Food Protect 1994; **57**: 1048–51.
33. Roberts D, Hooper W, Greenwood M, eds. *Listeria monocytogenes* and other *Listeria* spp, in: Practical Food Microbiology. London: Public Health Laboratory Service, 1995; 146.