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The occurrence of *Listeria* species in pâté: the Cardiff experience 1989

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SUMMARY

Samples of 216 pâtés were examined for the presence of *Listeria* spp. between May and August 1989; 35% were contaminated with *L. monocytogenes*, 5% of samples having counts of $> 10^4$/g. *L. innocua* was recovered from 2% of samples but no other species was isolated. Five serotypes of *L. monocytogenes* were represented (1/2, 4b, 4b(X), 4 not 4b or 4b(X), and NT), 4b was the predominant serotype and multiple serotypes were found in eight pâtés. The incidence of contamination with *L. monocytogenes* was greater in stored pâté (46%) than display pâté (30%). Sampling over a 21-day period showed an apparent increase in numbers of *L. monocytogenes* in 6 of 7 samples with multiple serotypes represented. There was no correlation between contamination of pâté by *L. monocytogenes* and the presence of coliforms. Comparisons are made between the contamination of soft cheese and pâté.

INTRODUCTION

L. monocytogenes has been isolated from a wide variety of foodstuffs [1], possibly reflecting its ubiquitous distribution in the environment. Increasing evidence suggests that listeriosis can be foodborne [2, 3]. Epidemiological data has supported an association with food [4, 5] and a limited number of outbreaks and sporadic cases of infection have been microbiologically traced to food vehicles [6, 7].

This association with food together with the ability of the bacterium to multiply at refrigerator temperatures has led to detailed examination of a number of foodstuffs including meat and meat products [8], salad vegetables [9] and dairy products, notably soft cheeses [10].

A chance finding in this laboratory in May 1989, suggested that pâté could be a potential source of *L. monocytogenes*. In view of the comparable shelf lives of pâté and soft cheeses a study was undertaken to determine the extent and level of contamination of pâté by *Listeria* spp. An interim report on the early stages of this study has already been produced [11]. A limited study to determine the extent of growth of *L. monocytogenes* in refrigerated pâté was included. Coliform counts were performed to establish any possible association with contamination with *Listeria* spp.

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MATERIALS AND METHODS

Pâté samples

Samples of pâté were obtained from retail and distribution outlets throughout South East Wales between May and August 1989. The majority were cut slices from bowls or loaves from delicatessen display counters or unopened packets from refrigerated storage though some vacuum packed individual portions were also tested. Most pâtés were imported and a number of varieties and manufacturers were represented. Seven vacuum-packed 2 kg pâté loaves were kept refrigerated (4–8 °C) and sampled repeatedly over a 21-day period to reflect the shelf life of the product.

Detection and enumeration of coliforms

Decimal dilutions of homogenized pâté were prepared in $\frac{1}{4}$-strength Ringer's solution (Oxoid BR52). Coliform counts were performed by a Miles and Misra surface drop technique on MacConkey Agar (Oxoid CM7) (limit of sensitivity $\geqslant 20$/g).

Detection and enumeration procedures for Listeria *spp.*

All samples were examined by a modification of the US Food and Drug Administration procedure using pre-enrichment. Twenty-five grams of pâté was added to 225 ml of buffered peptone water and incubated at 30 °C for 24 h. Ten millilitres was then added to 90 ml of Enrichment Broth made to the specifications of Lovett and co-workers [12]. After 24 and 48 h incubation at 30 °C the broth was plated onto Listeria Selective Agar (LSA, Oxoid CM856+SR140). LSA plates were incubated aerobically at 37 °C for 48 h. Numbers of *Listeria* spp. were estimated in pâtés found to contain the bacterium on enrichment. Decimal dilutions of homogenized samples used for coliform counts were stored at −20 °C and Miles and Misra surface drop counts performed on LSA (limit of sensitivity $\geqslant$ 20/g). In the latter part of the study it was found to be advantageous to perform counts at the same time as enrichment.

A proportion of æsculin hydrolysing colonies (up to 4) were subcultured onto 5% horse blood Columbia Base Agar (Oxoid CM331) for purity and differential biochemical tests were performed. The isolates were identified according to the criteria of McLauchlin [13]. Following storage at −80 °C in the 'Protect Bacterial Preserver' system (Technical Services Consultants TS70) the strains identified as *L. monocytogenes* were forwarded to Dr J. McLauchlin at the Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory for serotyping.

RESULTS

L. monocytogenes was isolated from 75 (35%) of 216 samples of pâté (Table 1). Both slices cut from loaves on display and portions of whole vacuum-packed pâtés from refrigerated storage were found to be contaminated. Although the majority of counts were < 20/g, 10 of the pâtés (9 from display, 1 from store) yielded counts of $> 10^4$/g. *L. innocua* was isolated from a further five display pâtés (counts of

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Table 1. *The incidence and counts of* Listeria monocytogenes *isolated from pâté by source*

Count of *L. monocytogenes* (/g)	Display ($n = 155$)	Store ($n = 50$)	NK ($n = 11$)	Total ($n = 216$)
< 20	21	17	4	42
20–100	4	2	0	6
$> 10^2$–10^3	4	3	0	7
$> 10^3$–10^4	4	0	0	4
$> 10^4$	9	1	0	10
ND	4	0	2	6
Total	46	23	6	75

ND, not performed; NK, not known.

Table 2. *Serotypes of* L. monocytogenes *isolated from pâté on first sampling*

Serotype	Number of isolates
1/2	4 (5%)
4b	52 (63%)
4b(X)	24 (29%)
Non-typable	2 (2%)
Not typed	1 (1%)
Total	83*

* Eight were contaminated by both serotype 4b and 4b(X).

$< 20 = 3$, $> 10^2 - 10^3 = 1$ and ND = 1) but no other species was isolated. No sample yielded more than one species. The contamination rate of pâté from store (46%) was higher than those on display (30%) though the proportion of isolates obtained by enrichment only (< 20/g) was notably higher in pâté from store, 74% compared with 50% from displayed pâté. The results of serotyping of isolates of *L. monocytogenes* are shown in Table 2. The predominant serotype was 4b followed by 4b(X). Eight samples were contaminated with more than one serotype.

Of the 183 samples of pâté examined for coliforms, 31% were found to be contaminated (Table 3). There was no correlation between the presence of coliforms and *L. monocytogenes*. Coliforms were recovered from 27 of 65 samples yielding *L. monocytogenes* and from a further 30 of 118 samples from which *L. monocytogenes* was not isolated.

Repeated sampling over 21 days of seven 2 kg pâté loaves showed variable results in terms of the counts of *L. monocytogenes* (Table 4). On initial sampling only two pâtés were found to be contaminated. Subsequent sampling resulted in *L. monocytogenes* being recovered from each of the pâtés after 7 days. The level of contamination was time dependent, with the exception of one pâté the count of *L. monocytogenes* appeared to increase with time. In order to determine whether distribution differences were a factor, two pâtés were sampled at three disparate sites on Day 9. The counts in pâté 2 were 240, 200 and 180/g and in pâté 5 were 3×10^5, $1{\cdot}3 \times 10^5$ and $6{\cdot}2 \times 10^4$. Serotyping of isolates of *L. monocytogenes* from these pâtés showed that the serotypes first isolated usually persisted over the 21-day period. Multiple serotypes were again detected, pâté 5 yielded four

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Table 3. *The incidence and counts of coliforms isolated from pâté*

Counts of coliforms (/g)	Number of pâtés ($n = 183$)
20–100	16
$> 10^2$–10^3	14
$> 10^3$–10^4	6
$> 10^4$–10^5	9
$> 10^5$–10^6	10
$> 10^6$	2
Total	57

Table 4. *Counts of* L. monocytogenes *in refrigerated pâté over a 21-day period*

	Time in days								
Pâté	1	2	3	5	7	9	12	15	21
1	NI	NI	NI	< 20	NI	< 20	< 20	< 20	140
2	160	NI	2×10^4	180	7×10^4	240	< 20	< 20	80
3	NI	NI	< 20	< 20	20	80	20	< 20	300
4	NI	NI	NI	NI	< 20	< 20	400	< 20	< 20
5	80	80	8×10^3	1×10^6	1×10^8	3×10^5	1×10^8	2×10^6	2×10^8
6	NI	NI	NI	NI	< 20	< 20	40	560	< 20
7	NI	NI	NI	NI	< 20	< 20	< 20	< 20	240

NI, Not isolated in 25 g.

different serotypes over the 21-day period including a strain of serotype 4 not 4b or 4b(X). The predominant serotypes were 4b and 4b(X). No other species of listeria was isolated.

DISCUSSION

The World Health Organisation Informal Working Group on Foodborne Listeriosis consider the primary means of transmission of human listeriosis is through the consumption of foodstuffs contaminated during production and processing [2]. Outbreaks of listeriosis have been attributed to coleslaw [14], milk [4] and soft cheese [6, 15], foodstuffs generally consumed without reheating [16]. Meat and poultry products are yet to be implicated in outbreaks of infection [17–19], though epidemiological evidence has associated undercooked chicken and uncooked hot dogs as risk factors in cases of sporadic listeriosis [5]. Sporadic cases are rarely linked with the consumption of any particular foodstuff [16] though the investigation of such cases remains problematic [20]. Occasional cases have however been microbiologically linked to a variety of foodstuffs [21, 22] including poultry [7] and frankfurters [23]. Serotyping and phage typing of strains of *L. monocytogenes* has indicated that previously unrecognized common source outbreaks may have occurred [24, 25] though their association with food remains to be determined.

The finding of *L. monocytogenes* in 35% of the samples of pâté is a cause for concern. The 5% of samples with counts of $> 10^4$/g are comparable with those demonstrated in soft cheese and considered 'unacceptable in a ready-to-eat food normally stored at a temperature at which the organism grows readily' [10]. *L.*

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monocytogenes has been isolated from up to 60% of raw chickens [10] though comparisons between studies are often hampered by the lack of a standardized methodology [26]. The incidence of *L. monocytogenes* in raw fresh and ground red meat varies between 0 and 92% [8] with reported numbers ranging from less than 20 to 10^3/g [2, 8]. Up to 30% of raw ready-to-eat products may contain *L. monocytogenes* though quantitative studies are lacking [2]. A survey of pre-cooked, ready-to-eat poultry showed that 12% were contaminated with *L. monocytogenes* whilst 18% of chilled meals, predominantly poultry, were similarly contaminated [27]. The level of contamination was however low (less than 100/g).

The presence of *L. monocytogenes* in pâté is due to either inadequate cooking or post-processing contamination. Controversy surrounds the heat-resistance of *L. monocytogenes* in milk and its ability to survive pasteurization, [26]. The thermotolerance of the bacterium in other foodstuffs was an area highlighted by the WHO Working Group [2] as being suitable for future study. Glass and Doyle [17] have shown that reprocessing pepperoni after drying by heating it to an internal temperature of 51·7 °C for 4 h rendered the product free of viable *L. monocytogenes*. Increasing evidence suggests that the presence of *L. monocytogenes* in food is a result of post-processing contamination from environmental sources [2, 5] with raw meat and poultry representing a potential source of cross infection [19]. The tolerance of *L. monocytogenes* to sodium chloride and sodium nitrate make the bacterium of particular concern as a post processing contaminant of refrigerated food [13]. Glass and Doyle [18] have stressed both the importance of preventing post processing contamination of ready-to-eat meat products and the limitations of refrigeration at 4–7 °C as a form of pathogen control. The isolation of *L. monocytogenes* from vacuum-packed pâtés in our study suggests that contamination occurred at some stage during manufacture though cross contamination between displayed pâtés could not be excluded as utensils were often shared between items. This emphasises the importance of basic hygiene procedures.

Comparison of the findings in soft cheese and pâté is instructive. The materials and methods of Pini and Gilbert [10] differ from those employed in this study though both are based on a modification of the US Food and Drug Administration procedure. Pre-enrichment and the larger samples tested in this study suggest the methods employed could be more sensitive than those of Pini and Gilbert [10]. The 35% of pâtés contaminated with *L. monocytogenes* compares with a figure of 10% for soft cheeses. With respect to the levels of bacterium, the 4·6% of pâté samples with counts of $> 10^4$/g are comparable with 4·5% of soft cheeses with similar levels. A major difference between pâté in this study and the findings in soft cheese of Pini and Gilbert is in the serotypes of *L. monocytogenes* isolated. Serotype 4b represented 63% of isolates from pâté but only 35% of those isolated in soft cheese whilst serogroup 1/2 represented 5% of isolates from pâté and 62% from cheese. Serotype 4b is responsible for the majority of cases of human listeriosis whilst representing a minority of isolates from food [8, 10]. Multiple serotypes in single products were found in both studies but the isolation of strains of serovar 4b(X) is interesting as this strain was responsible for a highly unusual cluster of 23 cases of listeriosis during 1987 in which no common risk factors were identified [25].

Pini and Gilbert [10] have shown distribution differences in the levels of *L. monocytogenes* in soft cheese possibly due to the effects of microenvironmental differences such as pH. The seeding experiments of Glass and Doyle [18] have shown the growth of *L. monocytogenes* in processed meat products to be largely product and pH dependent. Lactic acid bacteria may inhibit the growth of *L. monocytogenes* by reducing the pH as a result of the fermentation of available carbohydrate [18]. This may explain the lower levels of *L. monocytogenes* in fermented sausage products when compared with non-fermented ready-to-eat cooked meats [2]. Breer and Schopfer [28] suggest that the levels of listeria in meat are stable or decline and emphasise the 1000-fold lower counts found in meat. This study has shown that the levels of *L. monocytogenes* in pâté may increase with time allowing for possible distribution differences. The counts of up to $> 10^8$/g found in some pâtés are equivalent to the levels found in foods proven as the vehicles of infection [22, 29]. The absence of a correlation between contamination of pâté with *L. monocytogenes* and coliforms accords with the findings of Pini and Gilbert [10] who found no correlation between the contamination of soft cheese by *L. monocytogenes* and *Escherichia coli*. However, the presence of coliforms in 31% of a cooked product is further cause for concern.

Pâté has yet to be associated with cases of listeriosis and extreme caution should be practised in extrapolating the dangers of *L. monocytogenes* in food from experimental observations [13]. The extent of foodborne transmission remains uncertain though common-source outbreaks may remain unrecognized in the absence of unusual circumstances [25]. The high levels of contamination found in pâté and its extended shelf life together with the predominance of strains of serotype 4b suggest that pâté may be a significant source of the bacterium. Following the findings described here, co-ordinated surveys by the PHLS in July 1989 and July 1990 were carried out to further ascertain the extent of contamination of pâté with *L. monocytogenes* in England and Wales. These surveys showed a decrease in the quantitative and qualitative level of contamination between 1989 and 1990 (R. J. Gilbert, personal communication). In addition, following warnings from the Department of Health in July and August 1989, a decrease in the incidence of human listeriosis occurred in Britain (S. M. Hall, personal communication). The results of these surveys plus further evidence revealing a possible link between cases of human listeriosis and the consumption of pâté will be published elsewhere (J. McLauchlin, personal communication). Much, however, remains to be elucidated of the pathogenesis and epidemiology of listeriosis.

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