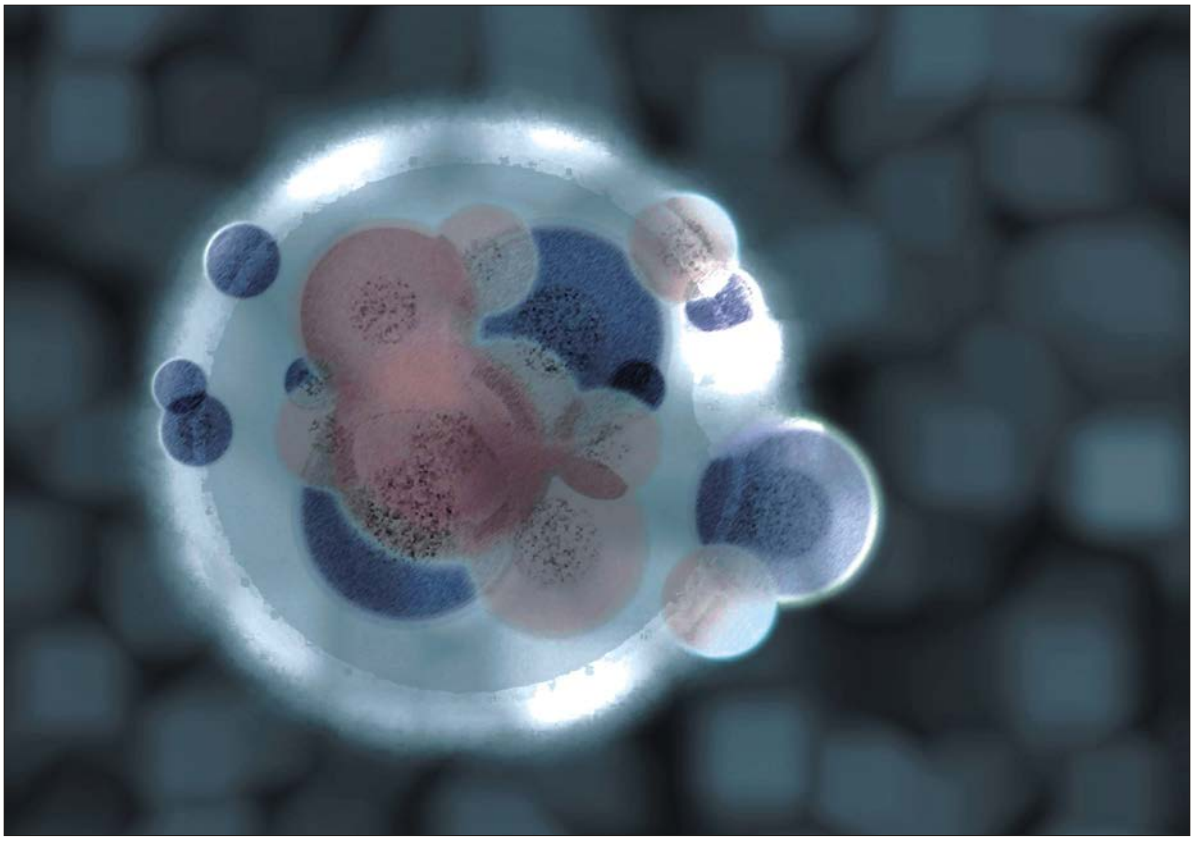

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Genetic Comparison of Isolates of *Listeria monocytogenes* and *Listeria innocua* from Different Sources in Australia

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Abstract: *Listeria innocua* is a closely related nonpathogenic species of *Listeria*, which has been suggested as an appropriate epidemiological marker for *Listeria monocytogenes*. Little information is available on the comparative epidemiology and molecular biology of *Listeria monocytogenes* and *Listeria innocua*. Using multilocus enzyme electrophoresis to analyse 168 strains collected mainly in poultry production environments, it was shown that *Listeria innocua* maintains 1.9 alleles per polymorphic loci and a genetic diversity of 0.12 compared with 2.9 alleles and a genetic diversity of 0.48 in *Listeria monocytogenes*. The number of Electrophortic Type (ET) found in isolates obtained from two poultry production plants in Sydney, Australia also varied, with 54 ETs in 73 isolates of *Listeria monocytogenes* and 10 ETs in 95 isolates of *Listeria innocua*. There was one common ET (67%) in *Listeria innocua* but no common ET in *Listeria monocytogenes*, where 79% of ETs were represented by a single isolates. Nine isolates obtained from episodic human cases of listeriosis in Australia represented nine different ETs and there was no evidence of a disease specific strain. The marked difference in the level and distribution of genetic variation in *Listeria innocua* compared to *Listeria monocytogenes* raises serious doubts as to the validity of using it as a marker surrogate for *Listeria monocytogenes*.

Key words: *Listeria monocytogenes*, *Listeria innocua*, multilocus electrophoresis, genetic comparison, poultry, food

INTRODUCTION

The ubiquitous nature of *Listeria* sp. results in contamination of numerous food products (Farber and Peterkin, 1991; Dhanashree *et al*., 2003; Vitas and Garcia-Jalon, 2004; Yucel *et al*., 2004). It has been also shown by epidemiological studies that listeriosis is a foodborne disease (Schlech *et al*., 1983; Baek *et al*., 2000; Finaly, 2001; Basti *et al*., 2006) and a number of foodborne outbreaks and sporadic cases of listeriosis have been reported, mostly in North America and Europe (Cox *et al*., 1989; Lovett, 1989). Food implicated in outbreaks of listeriosis have included various types of products such as dairy, meat, vegetable and sea food (Bell and Kyriakides, 2005; Schlech, 2000).

The pathogen *L. monocytogenes* and closely related species *L. innocua* occur at high incidence rates in poultry products (Jalali *et al*., 1996; Gibbons *et al*., 2006; Gudbjornsdottir *et al*., 2004; Mena *et al*., 2004). Though little is known about the epidemiology of these species in poultry production environments, it has been suggested that the presence of *L. innocua* is a convenient surrogate that can be used to detect the presence of *L. monocytogenes* (Baily *et al*., 1989; Genigegorgis *et al*., 1989; Varabioff, 1990; Lawrence and Gilmour, 1994; Franco *et al*., 1995). This suggestion has been made because of the difficulty of detecting *L. monocytogenes* in the presence of *L. innocua*, due to the competitive advantage of the latter during enrichment of food sample (Curiale and Lewus, 1994). It is important, as a consequence, to identify the relationships between populations of the two species found in different parts of the food production system and the origins of the *Listeria* in the system.

In a previous study of the distribution of *Listeria* sp. in the broiler production environment the widespread presence of the bacterium was observed (Jalali *et al*., 1996). Therefore it is important to identify the

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relationships between isolates from different parts of the system and the route of transmission of *Listeria* in the system. A powerful discriminatory typing method therefore is needed that would provide information on the distribution and therefore movement of *Listeria* through the poultry environment from broiler farm to the end of the processing plant and consumer.

Multilocus Enzyme Electrophoresis (MEE) has been used successfully as a typing method to study genetic diversity in a natural population of *L. monocytogenes* (Piffaretti *et al*., 1989; Buncic *et al*., 2001; Harvey *et al*., 2004) and to document the link between clinical cases and contaminated food products as well as to rule out a single common source as the cause of an outbreak (Bibb *et al*., 1989; Norrung, 1992).

The purposes of the present study were to employ MEE: (1) To determine the genetic diversity amongst poultry isolates of *L. innocua* and *L. monocytogenes* from a number of Australian sources. (2) To employ information on that diversity to: (a) evaluate the use of *L. innocua* as an industrial marker for *L. monocytogenes*, (b) increase understanding of the origins of and means of transmission of *Listeria* in poultry production environment and (c) compare poultry isolates with those from clinical and other sources.

MATERIALS AND METHODS

Bacterial strains: A total of 168 isolates of *Listeria* sp. were examined. These isolates included of 95 strains of *L. innocua* and 73 strains of *L. monocytogenes* isolated from two poultry processing plants (A and B) located in Sydney, Australia and their supplier farms as described elsewhere (Jalali *et al*., 1996).

The strains of *L. monocytogenes* examined consisted of two litter (farm), 51 poultry tissue and poultry processing environment isolates, nine human clinical isolates, 11 dairy environment isolates and two food isolates one from each of NSW Health Department, Division of Analytical Laboratory (DAL) and a stock culture of University of Western Sydney Hawksbury (UWSH). The poultry isolates from processing plant A consisted of six neck skin isolates and 11 environmental isolates. The environmental strains consisted of one surface swab isolated from the receiving area, six surface swab isolates from the chilling area, three ice isolates and one glove isolate. Poultry isolates from processing plant B consisted of 22 isolates from neck skin obtained after chilling and one isolate from each of caeca and feathers. Three whole carcass rinse sample isolates, one feather isolate and four cloacal isolates obtained from processing plant B was also typed.

Multilocus Enzyme Electrophoresis (MEE): The main disadvantages of MEE raised by some researchers when typing large numbers of strains is that the method is time consuming and labour intensive. This observation may result from the fact that all previous MEE work for typing *Listeria* has been carried out using starch gel electrophoresis. This limitation was overcome in this study by cellulose acetate as the support medium. Richardson *et al*. (1986) has demonstrated the advantage and convenience of cellulose acetate over other electrophoresis media such as starch, polyacrylamide and agarose. Cellulose acetate has the great advantage over polyacrylamide of being nontoxic and it is the only medium using low voltages that gives results in less than an hour. Other major advantages are that this medium is easy to handle and requires only a small amount of sample (0.5 -2 μL) and stain (2-3 mL).

Many enzyme loci have proven useful in the epidemiological study on *L. monocytogenes* (Piffaretti *et al*., 1989; Bibb *et al*., 1990; Sutherland and Porritt, 1995; Avery *et al*., 1996; Buncic *et al*., 2001; Harvey *et al*., 2004). The seven enzymes used in this study, were chosen because they have been reported to be polymorphic for *L. monocytogenes* in previous studies and showed well defined, easy to read profiles on cellulose acetate gel using a single buffer system, thereby making the method more convenient in practice. Obviously, more ETs might have been defined by investigating a larger number of enzymes and by using other buffer systems, but if a more laborious and time consuming typing method had been used, fewer isolates could have been typed and, thus, the overall epidemiological information might have been less.

Preparation of enzyme extracts: Single colonies of the organisms were first cultured in 10 mL of Brain Heart Infusion Broth (BHIB, Amyl media) which was then incubated at 37°C for 24 h. The culture was transferred aseptically to 50 mL of BHIB and incubated for a further 24h at 37°C on a shaker. The broth was tested for purity by overnight culture at 37°C on 5% blood agar (Oxoid). Each culture was harvested by centrifugation at 1000 g for 10 min and then resuspended in 0.5 mL of lysing buffer (100 μL β-mercaptoetanol, 10 mg NADP and 100 mL distilled water) (Richardson *et al*., 1986). Cells were broken up by applying three, 1 min cycles of sonication with a 100W Cole-Parmer sonicator microtip in an ice-water bath. The sonicated suspensions were centrifuged at 13000 RPM at 4°C for 20 min and the supernatant aliquoted into three eppendorf tubes and stored at -70°C until used for electrophoresis.

Sample application and gel electrophoresis: Aliquots of each isolate were applied to 10×17 cm cellulose acetate gels (Cellogel: Chemetron, Milan) and subjected to electrophoresis as described by Richardson *et al.* (1986) and Pupo and Richardson (1995). After preliminary investigation, 0.1 M tris-maleate buffer (100 mM tris, 4 mM maleic acid pH 7.8) was selected as the running buffer. Electrophoresis was performed in a cool room. A constant voltage of 10 V cm and a run time of 45 min were used.

Staining for enzymes: The enzymes detected were as follows: 6- phosphogluconate dehydrogenase (EC 1, 1, 1, 44, 6PGD), glucose 6-phosphate dehydrogenase (EC 1, 1, 1, 49, G6PD), alanine dehydrogenase (EC 1, 4, 1, 1, ALA), mannose phosphate isomerase (EC 5, 3, 1, 8, MPI), phosphoglucose isomerase (EC 5, 3, 1, 9, PGI), phosphoglucomutase (EC 2, 7, 5, 1, PGM), purine nucleoside phosphorylase (EC 2, 4, 2, 1, NP), peptidase-leucyl-leucyl-glycine (EC 3, 4, 11 or 13 LGG). Most of the enzymes assayed in the present study had been reported to be polymorphic for *L. monocytogenes* (Sutherland and Porritt, 1995; Bibb *et al*., 1989, 1990; Boerlin *et al*., 1991; 1992; Boerlin and Piffaretti, 1991). Stains were made up as given in Richardson *et al.* (1986). Relative mobilities were determined visually against two standard samples run on each gel. The relative mobility was established by scoring the relative anodal migration distance, i.e., enzymes nearest to the anode were given the highest score. All gels were scored by two individuals to avoid misscoring.

Analysis of data: Each combination of electromorphs over the set of loci studied was assigned to an Electrophretic Type (ET).

Genetic diversity (h) was determined using the formula:

$$h = \frac{(1 - \sum x_i^2)n}{n - 1}$$

where:

x_i = The frequency of the

i^{th} = Allele and n is the number of strains (Selander *et al*., 1986).

RESULTS

Genetic diversity: The number of alleles and genetic diversity at seven enzyme loci among Ets of *L. monocytogenes* are shown in Table 1. On average three alleles were found per locus. In contrast, only five of the nine enzymes assayed for *L. innocua* were polymorphic, with between two and three alleles per locus (Table 1). The number of alleles and genetic diversity at nine enzyme loci among ETs of *L. innocua* are shown in Table 2. It is clear that there is greater genetic diversity in the *L. monocytogenes* strains than in the *L. innocua* strains studied.

Table 1: No. of alleles and genetic diversity (h) at enzymes loci of *L. monocytogenes*

Enzymes	No of alleles	Frequency	Genetic diversity (h)
MPI	3.00	0.3169	0.6935
ALA	4.00	0.5368	0.4704
G6PD	3.00	0.3767	0.6319
6PGD	3.00	0.4165	0.5916
LGGF	2.00	0.7995	0.2032
LGGS	4.00	0.3451	0.6639
PGM	3.00	0.4240	0.5840
Mean	3.14	-	0.5483

Table 2: No. of alleles and genetic diversity (h) at enzyme loci of *L. innocua*

Enzymes	No. of alleles	Frequency	Genetic diversity (h)
MPI	3.00	0.6079	0.3962
ALA	3.00	0.8606	0.1408
G6PD	1.00	1.0000	0.0000
6PGD	1.00	1.0000	0.0000
PGM	2.00	0.9591	0.0413
PGI	2.00	0.7060	0.2971
LGGF	1.00	1.0000	0.0000
LGGS	2.00	0.9790	0.0212
NP	1.00	1.0000	0.0000
Mean	1.78	-	0.0996

Genetic relationships among strains: Fifty four ETswere identified among the 73 *L. monocytogenes* isolates studied. The ET designation and the enzyme profile of each ET are indicated in Table 3. In marked contrast, only ten ETs of *L. innocua* were detected among 95 isolates. The ET designation and the enzyme profile of each ET are shown in Table 4.

The genetic relationships among strains in relation to sources: The source of isolates of *L. innocua* and the distribution of different ETs in the two processing plants (A and B) at the three major stages of processing (post defeathering, post eviscerating, post chilling) and in the plant environment are shown in Table 5. ET 1 was the common form found in all situations with 64% of all the strains examined of this type. Within the *L. innocua* clones isolated from processing plant A, ET 1 constituted 59% of the strains, compared to 75% amongst strains isolated from the processing plant B. ET 1 was also the most frequent type in all three major stages of both processing plants as well as in the environmental isolates from processing plant A. The second most common type, ET 7, was found in 14% of all of the strains examined (15 and 12% of isolates from processing plants A and B, respectively). The frequency of these common ETs were found to be significantly different ($p = 0.05$) in the two

Table 3: ET and enzyme profile of *L. monocytogenes* isolates

			Enzyme relative mobility						
ET	Serotype	No. of isolates	MPI	ALA	G6PD	6PGD	LGGF	LGGS	PGM
1	1	2	2	3	5	2	3	2	2
2	4	1*	2	3	5	2	3	2	3
3	1	2	5	3	5	2	3	2	2
4	1	1	3	3	5	3	3	2	2
5	1	1	2	3	3	2	3	5	2
6	1	1	2	2	3	2	3	2	2
7	1	1	5	3	5	2	3	2	5
8	1	1	5	3	5	3	3	2	2
9	1	1	3	3	3	2	3	2	2
10	1	3	0	3	5	3	3	2	2
11	4	1	5	3	5	2	3	0	2
12	1	2	2	3	3	2	3	5	2
13	1	2	2	2	3	2	3	5	2
14	1	3	3	3	3	3	3	2	2
15	1	1	3	3	5	3	3	3	2
16	4	1	2	2	3	2	3	3	2
17	1	1	0	3	5	3	3	2	3
18	1	1	5	3	0	3	3	2	2
19	1	3	2	2	2	2	3	5	2
20	1	5	5	3	5	3	3	2	3
21	1	1	2	3	2	2	3	5	3
22	1	3	5	3	3	3	3	2	2
23	1	1	3	3	5	3	3	2	3
24	n	1	2	3	3	2	3	3	2
25	1	1	5	3	0	2	3	2	3
26	1	1	5	3	0	3	3	2	2
27	1	1	5	3	5	5	3	3	2
28	1	1	2	2	3	3	3	5	2
29	1	1	5	3	0	5	3	2	0
30	4	1	3	2	5	3	3	3	2
31	4	1	3	2	3	3	3	2	2
32	4	1	5	3	0	3	3	3	2
33	4	2	2	2	2	2	3	3	3
34	1	1	5	3	5	3	3	3	3
35	1	1	3	3	2	2	3	3	3
36	1	1	5	1	5	3	3	3	2
37	1	1	5	3	2	3	3	3	2
38	1	1	5	3	3	3	3	2	0
39	1	1	5	3	0	5	3	3	0
40	1	1	3	3	0	3	3	2	3
41	1	1	5	3	5	2	0	0	3
42	1	2	5	3	0	3	3	2	2
43	1	1	5	3	5	3	2	2	5
44	1	1	5	2	5	3	3	3	2
45	4	1	2	5	3	2	2	3	2
46	1	1	5	3	5	5	3	3	3
47	4	1	2	4	3	2	2	3	5
48	1	1	3	2	3	3	3	5	2
49	n	1	2	5	2	2	2	3	5
50	1	1	0	2	3	5	2	0	2
51	1	1	3	2	2	2	3	5	3
52	1	1	5	1	3	3	3	3	2
53	n	1	3	2	2	3	3	6*	2
54	1	1	5	2	5	3	3	0	2

*: The relative mobility was established by scoring the relative anodal migration distance, i.e., enzymes nearest and farthest to the anode were given the highest (6) and lowest (1) score

processing plants. The remaining ETs were found rarely. ET 4, 5, 6 and 9 were found in processing plant B, while ET 10, was isolated only from processing plant A. The remaining ETs (ETs 1, 2, 3, 7 and 8) were present in both processing plants (most of the strains of ET 8 was found in processing plant A).

Most of the ETs (80%) among the 73 *L. monocytogenes* clones isolated from poultry, dairy and humans were represented by only one isolate. Six ETs (11%) were represented by two isolates while four (7%) ETs were represented by three isolates. Only one (2%) ET (ET number 20) was represented five isolates. The two

Table 4: ET and enzyme profile of *L. innocua* isolates

	Enzyme relative mobility								
ET	MPI	ALA	G6PD	6PGD	PGI	LGGF	LGGS	PGM	NP
1	4*	4	4	4	4	4	4	4	4
2	4	3	4	4	4	4	4	3	4
3	3	4	4	4	4	4	4	4	4
4	4	3	4	4	4	4	4	4	4
5	4	4	4	4	3	4	4	4	4
6	4	1*	4	4	4	4	4	4	4
7	3	4	4	4	3	4	4	4	4
8	5*	4	4	4	4	4	4	4	4
9	4	3	4	4	3	4	4	4	4
10	4	4	4	4	4	4	3	4	4

*The relative mobility was established by scoring the relative anodal migration distance, i.e., enzymes nearest and farthest to the anode were given the highest (5) and lowest (1) score

Table 5: The distribution of the ETs within subpopulation of *L. innocua* isolates from different sources

		ETs No. (%)									
Source	Total No.	1	2	3	4	5	6	7	8	9	10
ns1 B	9	9	0	0	0	0	0	0	0	0	0
ns2 B	10	10	0	0	0	0	0	0	0	0	0
ns3 B	13	9	0	1	0	0	0	3	0	0	0
ns1 A	2	2	0	0	0	0	0	0	0	0	0
ns2 A	3	1	0	0	1	0	1	0	0	0	0
ns3 A	9	7	0	0	0	2	0	0	0	0	0
f3 A	1	0	0	0	0	0	1	0	0	0	0
sw1 A	4	2	0	0	0	1	0	1	0	0	0
sw2 A	1	1	0	0	0	0	0	0	0	0	0
sw3 A	9	6	1	0	0	0	0	2	0	0	0
rwcg A	5	3	0	0	0	0	1	0	1	0	0
cwo A	8	4	0	0	0	0	0	1	3	0	0
gloveA	4	1	0	0	0	0	0	3	0	0	0
ice A	5	1	0	0	0	0	0	1	2	1	0
ce B	3	1	1	1	0	0	0	0	0	0	0
cl 93 B	2	1	0	0	0	0	0	0	1	0	0
f 93 B	3	2	0	0	0	0	0	0	0	0	1
r 93 B	3	1	0	1	0	0	0	2	0	0	0
Plant A	51	28(59.0)	1(2.0)	1(2.0)	1(2)	3(6)	3(6)	8(15)	6(12.0)	1(2)	0.0
Plant B	44	33(75.0)	1(2.3)	2(4.6)	0	0	0	5(12)	1(2.0)	0	1(2.3)
Total	95	61(64.0)	2(2.0)	3(3.0)	1(1)	3(3)	3(3)	13(14)	7(7.0)	1(1)	1(1.0)

A: Processing plant AB: Processing plant B; ns: Neck skin samples; ce: Caeca samples; cl: Cloacal samples; f: Feather samples; poultry isolates in 1993; r93: Rinse sample from preliminary study; sw1;2 and 3: Surface swabs from area one, two and three rwcg: Recycling water for cleaning gutters; 1, 2 and 3: Stage one, two and three

Table 6: Distribution of *L. innocua* isolates ETs in different sampling occasion

Visit	No strain	ET1	ET2	ET3	ET4	ET5	ET6	ET7	ET8	ET9	ET10
V1, A	4	4	0	0	0	0	0	0	0	0	0
V2, A	4	2	0	0	1	1	0	0	0	0	0
V3, A	8	5	0	0	0	1	2	0	0	0	0
V1, B	14	12	1	1	0	0	0	0	0	0	0
V2, B	9	8	0	0	0	0	0	1	0	0	0
V3, B	11	8	0	1	0	0	0	2	0	0	0
V1,Env A	20	12	0	0	0	1	1	2	3	1	0
V2,Env A	16	6	1	0	0	0	0	6	3	0	0
93 B	8	4	0	1	0	0	0	1	1	0	1
94 B	1	0	0	0	0	0	0	1	0	0	0
total	95	61	2	3	1	3	3	13	7	1	1

A: Processing plant A; B: Processing plant B; En1: Environmental sampling trip one; En2: Environmental sampling trip two; V1: Visit one; V2: Visit two; V3: Visit three; 93 B and 94 B, Poultry isolates from processing plant B in 1993 and 1994

farm isolates grouped in one ET (ET 12). No particular relationship was found between ETs and sources of *L. monocytogenes* strains as the majority of ETs consisted of only one strain.

Distribution of ETs on different sampling occasions: Table 6 summarises the distribution of the Ets from *L. innocua* based upon sampling occasion. ET 1 was found to be present on all sampling occasions in both

processing plants. Four of the seven isolates from the preliminary study were also ET 1. The remaining ETs occurred only sporadically throughout the sampling period. In a marked contrast, all of the ETs from *L. monocytogenes* occurred only sporadically throughout the sampling period. Only one ET (ET number three) appeared more than once, on two sampling occasions in processing plant B.

DISCUSSION

In this study, the mean genetic diversity over the seven enzyme loci was found to be 0.5483 (Table 1). However, as loci known to be polymorphic were chosen, average diversity is of doubtful significance. Differences in number and source of isolates examined and the methods used, including the electrophoresis media, buffers and the kind and number of enzymes used make it difficult to compare this study directly with other studies. The finding of the present study, however is comparable to some of the previous studies. Bibb *et al.* (1989) examined 310 strains of *L. monocytogenes* and defined 56 ETs. Piffaretti *et al.* (1989) analysed 175 isolates and recovered 45 ETs. Some investigations (Piffaretti *et al.*, 1989; Bibb *et al.*, 1990) were of isolates from foodborne disease outbreaks. As these isolates are likely to be from the same clones, few ETsmight be expected. (Norrung and Gerner-Smith (1993) and Avery *et al.* (1996) have studied strains that were not isolated during known outbreaks of listeriosis and so would be expected to include a greater number of ETs. Most of the ETs (79%) in this study were represented by only one isolate. This figure is comparable with that obtained in previous studies by Avery *et al.* (1996), Piffaretti *et al.* (1989), Norrung and Skovgaard (1993) and Boerlin and Piffaretti (1991) who reported 67, 75, 72 and 66% of ETs to be represented by only one isolate, respectively.

In the present study, the isolates from nine sporadic human cases of listeriosis were from nine Ets and were no more closely related to one other than they were to other isolates. This is in contrast to other study Piffaretti *et al.*, (1989) and Norrung and Skovgaard (1993) where the majority of strains from cases of sporadic human listeriosis were classified into only two ETs. This may reflect the fact that only a small number of human clinical isolates from sporadic cases of listeriosis in Australia have been examined. This may also indicate that there is great diversity among clinical isolates of *L. monocytogenes* in Australia. In this study, eleven isolates from dairy factory environments represented seven ET. No relationship was observed between poultry, human and dairy isolates, except two ETs (ET 19 and 20) were represented by isolates from poultry and dairy environment and strains were not clustered by source (not shown). Although there is in general no epidemiological link between these sources, the presence of ET 19 and 20 in isolates from poultry and dairy sources may indicate that the possibility of cross contamination. Further epidemiological data would be necessary to test this possibility.

Neither of the litter isolates (ET 12) and nor the one caecum isolate (ET 11) matched ETs collected from other sources. This suggests that *L. monocytogenes* in the processing plant is unlikely to have arisen from the farm studied. These observations are in agreement with the findings of Boerlin and Piffaretti (1991) in a meat slaughterhouse that animal strains do not easily contaminate or survive and multiply in a processing environment. They are replaced by other strains which are presumably better adapted for survival and multiplication in such environments. However there is still the possibility that the very low percentage of incoming birds which are *Listeria* carriers might play a role in the dissemination of *Listeria* in processing plants. Because of the low level of incidence of *Listeria* sp. found on the farms (Jalali *et al.*, 1996), the number of farm isolates available for typing, were limited. Consequently it is not possible to rule out sporadic contamination of the processing plant from incoming poultry though it seems very unlikely to be the major source.

The 51 carcass isolates of *L. monocytogenes* analysed by MEE included 39 ETs. Most of the ETs (80%) were represented by only one isolate indicating that there is great diversity amongst *L. monocytogenes* isolates from Australian processing plants. From the epidemiological point of view, sporadic occurrence of large numbers of genotypes within the processing plants and changing throughout the sampling period, suggests that contamination is from a wide range of external sources. No particular relationship was found between Ets of *L. monocytogenes* strains and different sites within the poultry processing plants as the majority of ETs consisted of only one clone. However in both processing plants, many different ETs were detected at the same site on the same sampling occasion. For instance five, seven and five different ETs were detected in neck skin samples taken after chilling from processing plant B on visit one, two and three, respectively. The fact that genetically distinct strains can survive in this environment suggests that different strains can co-exist in the same site or that there is continual massive recontamination of the plant. It seems likely that many different strains of *L. monocytogenes* can survive in such an environment.

The collection of 95 poultry strains of *L. innocua* isolated during the course of the present study was

examined using MEE at nine enzyme loci. Four of the nine enzymes tested (G6PD, 6GPD, LGG,F and NP) were monomorphic, whereas two enzymes (MPI and ALA) each had three alleles and three enzymes (PGM, PGI and LGG,s) each had two alleles. Some other authors have also found that NP were monomorphic for *L. monocytogenes*. In contrast, G6PD and 6GPD were polymorphic for *L. monocytogenes* (Table 1) and appear to be monomorphic for *L. innocua*. PGI which has usually been reported as monomorphic for *L. monocytogenes* (Kolstad *et al*., 1992; Norrung 1992; Norrung and Skovgaard, 1993; Sutherland and Porritt, 1995; Avery *et al*., 1996) was found to have two alleles for *L. innocua*. These results indicate that for epidemiological or population genetic studies of *L. innocua* it is necessary to use a different set of enzymes compared to those used with *L. monocytogenes*.

In contrast to *L. monocytogenes*, which has large amounts of genetic diversity appearing in many combinations, the 95 *L. innocua* isolates separated into only 10 ETs (Table 4) and the mean genetic diversity over the nine enzyme loci was found to be 0.0996 (Table 2). A single common genotype (ET 1) was found in both processing plants though at different frequencies. This ET constituted 59 and 77% of the strains isolated from the processing plants A and B respectively. Most of the isolates from the preliminary study (five out of eight) isolated a year earlier were also classified in this type. ET 1 was also found to be the most frequent type in all three major stages in both processing plants as well as in the environmental isolates taken from processing plant A. The remaining ETs (2-10) occurred only sporadically throughout the sampling period with strains specific to each of the processing plant being identified. Most of these types were found on no more than one sampling occasion suggesting continuing contamination from a wide range of sources.

The high prevalence of one or a small number of genotypes within both processing plants may be due to a number of factors. Firstly contamination within the plant may be a result of one or two strains occupying a niche in the processing chain. As a result of this, repeated contamination of poultry and subsequently of the processing environment occurs. Although *L. innocua* was not found at the farm level, it is possible that a limited number of farms may be supplying contaminated birds, with the *Listeria* subsequently being spread by cross-contamination from equipment within the plant. This view is supported by the isolation of ET 1 in cloacal, caeca and feather samples. This kind of contamination during processing has been shown with *Campylobacter jejuni* (Oosterom *et al*., 1983; Genigegorgis *et al*., 1986). Although the result of the previous study (Jalali *et al*., 1996) and another study (Husu *et al*., 1990) have shown that chickens are not likely to be a common reservoir for *Listeria*, sporadic *Listeria* carrier chickens faeces may still be the source of *Listeria* on the surface of the carcasses as well as in the processing plant environments. Ineffective sanitary operations at the processing plants may then turn this into a *Listeria* source.

The sporadic occurrence of most of the *L. innocua* ETs throughout the sampling period (Table 5) suggests that contamination is repeated and from a wide range of sources or selection of a particular ET. For instance ET 4 was found only at visit two of processing plant A and ET 2 was found on the first and second visits to processing plants B and A respectively (Table 5). There are a number of stages during processing where selective pressures may result in selection of a particular ET, then specific ETs may be selected during the various stages of processing prior to packaging e. g. scalding, defeathering, evisceration and chilling.

Alternatively the high prevalence of some genotypes may be maintained due to the ability of some strains to persist on environmental surfaces by means of surface attachment, or biofilm formation as it suggested by Archer (1990). In this study it was found that some strains of *L. innocua* persist longer than strains of *L. monocytogenes* in poultry processing environments and this may be due more to their ability for biofilm formation.

ET 7 and ET 8 were predominantly detected from environmental samples of processing plant A (Table 4 and 5). Neither of these ETs were found in poultry tissue samples in processing plant A. This suggests that environmental contamination is not the origin of the carcasses contamination by these ETs however, ET 7 was isolated from poultry samples in processing plant B. As environmental samples were not taken from this processing plant, it is difficult to substantiate any links between the two. However it is clear that these two ETs are adapted to such environmental niches.

Although *L. innocua* ET 1 was found to be common in both processing plants, there is a potential source of error which should mentioned. The fact that ET 1 occurs more frequently than other ETs suggests that this may be a very common genotype within poultry processing environments. Also the fact that this genotype was present throughout the period of the study, even in the preliminary study which had been carried out two years earlier, suggests that there is a common source of contamination or that it is a particularly persistent type within this environment. The other possibility is that the selective enrichment procedure which was used to isolate *isteria* sp. may lead to the suppression of certain clones and the selection of others thereby giving a false picture of the clonal distribution of the organisms.

The marked difference in the genetic structures of *L. monocytogenes* (i.e., different numbers of ETs and different rates of ET turnover) indicates that the two species either need different niches or, more likely *L. innocua* out compete *L. monocytogenes* at high temperatures but *L. monocytogenes* is able to compete with *L. innocua* at low temperatures (chilling stage). It would be of great interest to examine the competition abilities of the two species and of strains of the two species taken from different environments. It is also clear from the present study that there are marked difference in the biology of the two species and any attempt to use *L. innocua* as a surrogate for *L. monocytogenes* would need to demonstrate the validity of the assumed equivalence of two species.

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