Prevalence of Listeria monocytogenes in Retail Beef and Poultry

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ABSTRAK

Kajian awal ke atas daging lembu tempatan dan impot, daging lembu kisar, potongan-potongan ayam, daging ayam kisar dan organ-organ dalaman ayam daripada pasar-pasar basah dan pasaraya-pasaraya telah dijalankan dan dilakukan penganalisaan kehadiran Listeria monocytogenes. Pemencilan L. monocytogenes telah dilakukan dengan kaedah plating langsung di atas Agar Palcam dengan menggunakan pengkayaan pemilih dan pengkayaan sejuk pada 37° dan 4° C dalam kaldu pengkayaan L-Palcamy. Ayam sempurna serta perkakas-perkakas yang telah di gunakan semasa pemprosesan ayam juga dikesat (swab) dan di uji bagi kehadiran organisma tersebut. L. monocytogenes telah di kesan dalam 6 sampel daging lembu impot dan 3 sampel daging lembu tempatan. Daripada 16 sampel ayam yang diperolehi dari 4 pasar yang berlainan, 6 mengandungi L. monocytogenes. Sampel-sampel daging lembu dari pasaraya adalah negatif bagiL. monocytogenes tetapi organisma ini telah dikesan dalam 3 dari 4 sampel daging lembu kisar impot, dan 2 dari 4 sampel daging lembu kisar tempatan. L. monocytogenes juga telah didapati dalam 3 dari 4 sampel daging ayam; 2 dari 4 sampel daging ayam kisar dan 1 dari 4 organ-organ yang telah dikaji. Karkas-karkas ayam dari pasar A adalah negatif bagi L. monocytogenes tetapi ini tidak benar bagi karkaskarkas ayam dari pasar Boleh kerana 15 dari 24 sampelnya di dapati positif bagi L. monocytogenes. Tidak ada L. monocytogenes yang dapat dikesan daripada peralatan di pasar A, tetapi organisma tersebut dapat dikesan pada peralatan dari pasar B. Hasil-hasil ini mencadangkan bahawa daging-daging runcit yang telah diperoses selama beberapa jam lebih awal sesuai bagi pertumbuhan L. monocytogenes dan seterusnya meningkatkan silang.

ABSTRACT

Preliminary studies on local and imported beef, minced beef, chicken pieces, minced chicken meat and internal organs of poultry from wet markets and supermarkets were carried out and analysed for the presence of Listeria monocytogenes. L. monocytogenes was isolated by direct plating on Palcam agar and with selective and cold enrichment at 37° and 4° C in L-Palcamy enrichment broth. Whole chickens and the equipment used for processing of poultry were also swabbed and examined for the presence of the organism. L. monocytogenes was detected in 6/16 imported and 3/6 local beef samples. Out of 16 poultry samples which were obtained from four different wet markets, six contained L. monocytogenes. Beef samples obtained from supermarkets were negative for L. monocytogenes but the organism was detected in three of four imported minced beef, and two of four local minced beef samples L. monocytogenes was also detected in three of the four chicken samples; two of four minced samples and one of four organs which were obtained from supermarkets. Chicken carcasses from wet market A were negative for L. monocytogenes but this was not true for chicken carcasses from wet market B because 15 out of 24 were positive for L. monocytogenes. No. L. monocytogenes was detected from the equipment in wet market A, but equipment in wet market B was positive for the organism. These results suggest that retailing meats which have been processed for many hours favour growth of L. monocytogenes and also enhance cross-contamination.

INTRODUCTION

Listeria monocytogenes is a gram positive, nonsporing, aerobic to facultative anaerobic, psychrotrophic, rod-shaped bacterium which exhibits pathogenicity towards humans and other animals. L. monocytogenes is an opportunistic foodborne pathogen which can

cause abortion in pregnant women as well as meningitis in newborn infants and immunocompromised adults (Gray and Killinger 1966; Ralovich 1984; and Seeliger 1961).

Since 1983, more than 150 cases of listeriosis including at least 54 deaths in the USA resulted

from consumption of food containing the pathogen (El-Shenawy and Marth 1989). It has also been reported that the incidence of listeriosis in England and Wales has increased by 15% between 1986 to 1989 (Cox 1989).

Brackett (1988), reviewing the work of other investigators, summarized that *L. monocytogenes* is widespread in nature and can be isolated from a variety of sources such as poor quality silage, vegetation, soil, sewage, mud, slaughterhouse waste, milk of normal and mastitic cows and the faeces of healthy animals.

There has been an increase in surveillance of L. monocytogenes as a result of two large outbreaks of listeriosis, which resulted from the consumption of contaminated pasteurized milk (Fleming et al. 1985) and Mexican-style cheese (James et al. 1985). Surveillance has shown that L. monocytogenes can be present in both raw and pasteurized milk (Liewen and Plautz 1988; Garayzabal et al. 1987; and Lovett et al. 1987), soft and semi-soft cheese (Pini and Gilbert 1988; Beckers et al. 1987; and Faber et al. 1988) and in meat and poultry (Gitter 1976; Skovgaard and Morgen 1988 and Faber et al. 1989).

Presently, a review of local published literature revealed no reports on the prevalence of *Listeria monocytogenes* in foods in Malaysia. The objectives of this preliminary study are (1) to determine the presence of the organism in poultry and beef that are sold at local wet markets and supermarkets in Malaysia, and (2) to examine the equipment and utensils used for the processing of poultry at the wet market for presence of *L. monocytogenes*.

MATERIALS AND METHODS

Sampling

Five hundred g of both local and imported beef, minced beef, chicken pieces, minced chicken meat and internal organs (liver and gizzard) of poultry were purchased from 4 local wet markets: Kajang (A), Sungei Besi (B), Serdang (C) and Bandar Baru Bangi (D) and two supermarkets E and F.

Whole birds from wet markets A and B that had been freshly processed and dressed and those that were already processed and ready for sale were swabbed externally and internally. The whole external area of the bird was swabbed twice using different swabs. Similarly, the whole internal area of each bird was also swabbed twice. The bird was split open before the internal area was swabbed. The equipment used for processing chicken was swabbed. The area swabbed was approximately between 10-100 sq cm depending upon the size of the equipment. The swabs were then placed in L.

Palcamy enrichment broth (Van Netten et al. 1989). All samples obtained were transported to the laboratory in polystyrene containers containing ice.

Isolation of Listeria monocytogenes

Isolation of *L. monocytogenes* was carried out by plating on Palcam agar and with selective cold enrichment using L-Palcamy enrichment medium. The agar and enrichment medium were prepared according to Van Netten *et al.* 1989.

All samples except for the swabs were examined for L. monocytogenesusing three different methods. In the first method, 20 g of each sample was homogenized in a stomacher bag containing 180 mL of 1% peptone water (Oxoid) for 1-2 min using a stomacher (Colworth). 0.1 ml was spread plated on Palcam agar and incubated at 37°C. The second method involved placing 20 g of each sample in 300 mL Erlenmeyer flask which contained 100 mL of L-Palcamy enrichment medium and incubating the mixture at 37°C. The contents of the flask were later streaked on Palcam agar plates in triplicate after 24 and 48 h of incubation. The third method was similar to method II except that the sample-enrichment medium mixture was incubated at 4°C and streaked on the Palcam agar plates after 7, 14 and 21 d of incubation. The plates prepared using all three methods were then incubated at 37°C for 24-48 h.

The swabs were analysed in a similar manner to the other samples but with the exclusion of the first method described above. All the media used were obtained form Oxoid. The antibiotics and chemicals were purchased from Sigma except for Ceftazidine which was provided by Glaxo.

Samples were considered positive whenever presumptive *Listeria* colonies were isolated with any of the methods described above. The *Listeria* colonies were further characterized as described below.

Identification of Listeria monocytogenes

Presumptive positive *Listeria* colonies that were grey green with a black sunken center which exhibited black halo were picked up and further purified by restreaking on Tryptone soya agar (Oxoid). These were identified using API 20E system, (API International, S.A) Isolates that were gram (+), catalase (+), motile at 21°C (+), Methyl Red (+), β hemolysis (+) Rhamnose (+) were considered to be *L. monocytogenes*.

RESULTS

Results in Table 1 show that L. monocytogenes was present in imported beef obtained from four

TABLE 1

Analyses of local and imported beef from wet markets for the presence of *Listeria monocytogenes*

Market	Local/Imported Beef		No. of Samples Tested Positive
A	Imported	4	1
	Local	4	1
В	Imported	4	2
	Local	4	2
C	Imported	4	1
	Local	4	0
D	Imported	4	2
	Local	4	0

different wet markets. 50% (2/4) of the imported beef samples obtained from markets B and D were positive while only 25% (1/4) of the imported beef samples from markets A and C were positive. As for local beef, 50% (2/4) of the samples obtained from market B were positive while only 25% (1/4) of the samples obtained from market A were positive. All samples tested from markets C and D were negative for L monocytogenes.

Table 2 shows that 50% (2/4) of chicken pieces that were obtained from markets A, B and C were positive for *L. monocytogenes*, whereas samples from market D were negative. The organism was not isolated from any of the freshly processed birds

TABLE 2
Presence of Listeria monocytogenes in chicken pieces
obtained from local wet-markets

Market	No. of Samples	No. of Samples Tested Positive
A	4	2
В	4	2
C	4	2
D	4	0

from market A that were swabbed both internally and externally (Table 3). All the equipment from market A that was examined for L. monocytogenes was found to be negative (Table 3). However, the swabs from all the cages used for the transportation of live chickens gave positive results. Out of the four chicken organs from market A that were analysed, only one was positive for L. monocytogenes (Table 3).

Contrary to the findings obtained with the external and internal sites of the chickens from market A, L. monocytogenes was isolated from the external surface of 15 chickens and the internal site of 7 chickens from market B (Table 3). L. monocytogenes was also isolated from the floor, surfaces of chopping boards and tables. Baskets that were used to transport dressed chicken were negative for L. monocytogenes. 75% (3/4) of the organs examined were positive for L. monocytogenes (Table 3).

TABLE 3

Isolation of Listeria monocytogenes from chickens and equipment from markets (A) and (B)

Site of Swabbing	No. of Samples	No. of Samples Tested Positive from Market A	No. of Samples Tested Positive from Market B
External area of chicken	24	0	15
Internal area of chicken	24	0	7
Floor	4	0	2
Cages	4	4	NA [@]
Defeathering machine	'4	0	NA [@]
Chopping board	4	0	2
Table tops	4	0	2
Organs	4	1	3

[®] NA: Not analysed. (These items were not available at Market B)

TABLE 4				
Isolation of Listeria monocytogenes from meat and chicken obtained from supermarkets E and F				

Type of Samples	Supermarket	Imported/ Local	No. of Samples	No. of Samples Tested Positive
Beef		Imported	2	0
	E	Local	2	0
		Imported	2	0
	F	Local	2	0
Minced		Imported	2	1
Beef	E	Local	2	1
		Imported	2	2
	F	Local	2	1
Chicken	E	Local	2	2
	F	Local	2	1
Minced	E	Local	2	1
Chicken	F	Local	2	1
Chicken	E	Local	2	0
Organs	F	Local	2	1

Local and imported beef samples bought from the two supermarkets were free from L. monocytogenes (Table 4). This, however, was not the case for minced beef as 50% (1/2) of both the local and imported minced beef samples from supermarket E examined showed positive results, while all the imported minced beef samples and 50% (1/2) of the local minced beef obtained from supermarket Fwere positive for L. monocytogenes. With chickens, all the samples from supermarket E and 50% (1/2) of the samples from supermarket F were positive for L. monocytogenes. 50% (1/2) of the minced chicken samples from both supermarkets was found to be positive for L. monocytogenes. The organism was not present in chicken organs obtained from supermarket E but was detected in 50% (1/2) of the samples from supermarket F.

DISCUSSION

Results of this study show that *L. monocytogenes* is present in most of the animal products examined and in the processing environment. Similar findings have been reported by other investigators. Kwantes and Issac (1971) isolated *L. monocytogenes* from 57% of the fresh and frozen poultry sampled. Pini and Gilbert (1988) reported that 60% of raw chickens (fresh and frozen) were contaminated with *L. monocytogenes* and 25% with other *Listeria* spp. Elischerova (1976) isolated *L. monocytogenes* from 41.5% of meat surfaces examined. Examination of 113 raw meat samples in Italy revealed that 12% were contaminated with *Listeria*

spp.; 9 out of the 13 isolates were L. monocytogenes, with the remainder being L. innocua (Luppi et al. 1988). Johnson et al. (1990), after analyzing surveillance data of many investigators from different parts of the world, concluded that the prevalence of Listeria spp. on fresh meats can range from 0 to 68%, with pork being more commonly contaminated than beef or lamb. These authors also observed that the prevalence of Listeria in ground products and other products requiring cooking before consumption ranges from 8 to 92%. The presence of L. monocytogenes in slaughterhouse effluents further suggests that contamination of meat and poultry may be common (Watkin and Sleath 1981). Boyle et al. (1990) demonstrated that L. monocytogenes was capable of multiplication at both 8°C and 35°C, in waste fluids collected from the clean-up of a meat grinder, especially in the floor drain fluid.

At local wet markets in Malaysia freshly slaughtered beef and frozen beef (both imported and local) are sold at ambient temperature. Left-overs are either re-frozen or sold at night markets (pasar malam). In many markets, the bench tops are either wooden or made of concrete and the chopping boards are large blocks of wood. Poultry is either slaughtered and processed at the wet markets or slaughtered and processed elsewhere and then sold at these markets. If slaughtered and processed at the wet markets, then the birds are only slaughtered and processed when they are bought.

The incidence of L. monocytogenes in meat and meat products reported by various investigators is influenced by many factors including geographical differences, differences in animal-rearing, handling and slaughtering practices, and differences in food handling practices (including storage conditions and, especially, temperature control) (Johnson et al. 1990). The source of L. monocytogenes can be the meat or poultry itself, crevices in the benchtops and equipment such as knives or chopping boards. Retailing meat or dressed chicken at ambient temperatures for many hours will favour rapid growth of L. monocytogenes. Refreezing or chilling is not going to kill or retard the growth of L. monocytogenes as it is a psychrotrophic organism. Genigeorgis et al. (1989, 1990) reported that L. monocytogenes which was initially present on chicken and turkey parts such as wings, drumsticks and tails was able to grow and increase in number when stored at 4°C. As Listeria can be isolated from many environments (Brackett 1988) it is not surprising to observe that a large number of samples were positive. Results in Table 3 indicate that retailing chickens which have been processed much earlier at ambient temperatures for many hours will favour growth of L. monocytogenes and enhance cross-contamination. Chicken from market A which were freshly slaughtered and processed were negative for L. monocytogenes, whereas chicken sold at market B which were slaughtered elsewhere had a higher incidence of L. monocytogenes.

Chicken sold at supermarkets are slaughtered and dressed elsewhere. At the supermarkets, the chickens are sold whole, in the form of pieces or minced. Processing of chicken and beef into various cuts and grinding is done at the markets. The presence of L. monocytogenes in chicken, minced chicken and beef obtained from supermarkets suggests that either the chickens were contaminated when they arrived at the supermarket or there was cross-contamination during subsequent proces-sing. Genigeorgis et al. (1989) examined 160 packages containing drumsticks, wings and whole livers of poultry for the prevalence of Listeria spp from three different supermarkets. These investigators reported that the overall prevalence of L. monocytogenes in the skin of wings, drumsticks and livers was 10, 15 and 14%, respectively. These authors also reported that the prevalence of L. monocytogenes on the hands and gloves of persons hanging birds after chilling, cutting carcasses and packaging parts was 10% (2/20) 36.4% (4/11) and

45.5% (20/44) respectively. In another study involving turkeys, these authors reported that L. monocytogenes was detected in 20% (12/60) of the wings, 13.3% (8/6) of the drumsticks, and 11.7%(7/60) of the tails. The incidence of L. monocytogenes spp. on the hands and gloves of the persons hanging birds (turkey) after chilling, cutting carcasses, and packaging parts was 10% (3/30), 33.3% (10/30) and 16.7% (5/30), respectively (Genigeorgis et al. 1990). Bailey et al. (1989) examined ninety broiler carcasses which were obtained from retail stores. Listeria spp. was recovered from 34 of 90 (38%) of the carcasses sampled, while L. monocytogenes was recovered from 21 of 90 (23%) of the carcasses sampled. Faber et al. (1989) in a survey of retail foods in Canada reported that 9 of 16 (56.3%) chicken legs, 38 of 44 (86.4%) ground meats, and 6 of 30 (20%) fermented sausages contained L. monocytogenes.

The prevalence of L. monocytogenes in retail poultry and meat, as shown in this study and other studies cited in this article, may indicate an increased health risk, if not for the general "healthy" public, then for pregnant women, the elderly, the young, and immunocompromised individuals exposed to undercooked poultry meat. The ability of L. monocytogenes to survive in chicken breasts which were cooked to an internal end point temperature of 73.9°C and subsequent growth to initial levels during storage at 4 and 10°C have been reported (Carpenter and Harrison 1989). Several studies indicate that post-processing contamination of ready-to-eat products with L. monocytogenes may pose a hazard. Glass and Doyle (1989) observed growth of L. monocytogenesat 4.4°C in ham, bologna, sliced chicken and turkey products, wieners and fresh bratwurst; but little or no growth was observed on summer sausage or wart beef slices. Kerr et al. (1988) isolated L. monocytogenes from 5 to 21 samples of cook-chill food (product is cooked, rapidly chilled, refrigerated, then reheated before consumption); all isolations were from poultry dishes. Results of a monitoring programme of ready-to-eat meat products in the U.S. revealed that 5-12% of products sampled in 1987 and 10-13% products sampled in 1988 were contaminated with L. monocytogenes (Wilson 1989). Gilbert et al. (1989) isolated L. monocytogenes from 63 of 527 (12%) samples of ready-to-eat poultry, 13 of 74 (18%) chilled meals and 10 of 627 (2%) main course items from cookchill catering units.

In conclusion, the results of this preliminary survey and the work of other investigators emphasize the need for reducing the incidence of L. monocytogenes in foods. A recent case-control study attributed 20% of the risk of sporadic listerosis to the consumption of under-cooked chicken or uncooked hot dogs (Schwartz et al. 1988). From this study it is obvious that the relevant regulatory agencies need to review the way meat and poultry are sold at local markets. Meat and poultry should not be sold at ambient temperature and sales at night markets should be curtailed or controlled according to safety procedures. The presence of L. monocytogenesin foods might not be extremely hazardous to the "healthy" general public but does pose a serious health hazard to certain age groups. It is for these reasons that comprehensive surveys on the presence of L. monocytogenes in various types of foods should be carried out periodically.

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