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## OCCURRENCE OF *LISTERIA MONOCYTOGENES* IN FOOD IN SRI LANKA

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**Abstract:** A study on the occurrence of *Listeria monocytogenes* in market samples of different food items indicated that 38% of the samples contained *L. monocytogenes*. The percentage of positive samples was highest among vegetables (49%), second in chicken (34%) and lowest in dairy products (26%). Inactivation of *L. monocytogenes* during cooking of green leaves indicated that *L. monocytogenes* was recovered from samples upto 8 min of cooking by which time the temperature was 72°C. *L. monocytogenes* strains isolated had haemolytic activity of 16 to 64 minimal haemolysis units by the microhaemolysis test.

**Key words:** *Listeria monocytogenes*, food, heat inactivation.

### INTRODUCTION

*Listeria monocytogenes* is a gram positive, motile, non-sporeforming rod which is ubiquitous in nature. It causes severe infections in man, with case fatality rates of 11% under forty years of age and 63% over 60 years of age.<sup>1</sup> This organism is capable of slow multiplication at refrigeration temperatures<sup>2</sup> and it is tolerant to preserving agents such as 16-20% sodium chloride<sup>3,4</sup> and 120 ppm sodium nitrite.<sup>5</sup> On the basis of microbiological and epidemiological evidence, a wide range of foods have been associated with human listeriosis; e.g. raw or undercooked chicken,<sup>6</sup> pasteurized milk,<sup>7</sup> raw vegetables<sup>8</sup> and soft cheese.<sup>9</sup> This has led to a growing interest in surveying food for the presence of *L. monocytogenes*. Its presence in food or food-borne outbreaks of listeriosis have not been reported in Sri Lanka.

The objective of the present study was to determine the presence of *L. monocytogenes* in certain food items which have been implicated as sources of food-borne listeriosis in other countries. A preliminary study carried out to determine the efficiency of the isolation technique indicated that some green leaves, gotukola (*Centella asiatica*) and mukunuwanna (*Alternanthera sessilis*) which are used as vegetables, are highly contaminated with *L. monocytogenes*. Therefore the present study also determined its survival during partial cooking of green leaves in the preparation of mallun (a local preparation made by briefly heating vegetables and grated coconut).

Since haemolysis of *L. monocytogenes* has been identified as a virulence factor,<sup>10</sup> the study aimed also to determine the haemolytic activity of local strains isolated.

## METHODS AND MATERIALS

*Sampling:* A total of 139 samples of food which included fresh and frozen chicken, vegetables and dairy products were obtained from retail shops in Colombo, Dalugama, Gampaha and Kiribathgoda. The retail outlets from which the samples were obtained included supermarkets, farm shops, groceries, milk booths and public markets.

*Isolation of L. monocytogenes:* A 25 g sample of food was homogenized and incubated in *Listeria* enrichment broth.<sup>11</sup> After 24 h of incubation, broth was plated on to *Listeria* selective agar.<sup>12</sup> Typical colonies were subcultured on nutrient agar and identified using the criteria of McLauchlin (1987).<sup>13</sup> The morphological characters used in identification were gram positive, non-sporing, non-capsulated, non-pigmented and non-acidfast features of the isolates. The biochemical tests used in identification were catalase, oxidase, methyl red and Voges-Proskauer tests, haemolysis on sheep blood agar, CAMP test with *Staphylococcus aureus* and *Rhodococcus equi*, fermentation of carbohydrates (adonitol, arabinose, glycogen, mannitol, xylose, L-rhamnose, D-glucose, trehalose and salicin), indole production, nitrate reduction and gelatin, aesculin and casein hydrolysis. *L. monocytogenes* ATCC 19111 obtained from the Czechoslovakian Culture Collection (National Institute of Public Health, Czechoslovakia) served as a reference strain.

*Quantitation of haemolytic activity (Microhaemolysis test):* The microplate technique<sup>14</sup> was performed on all isolates to quantitate the haemolytic activity due to listeriolysin. Minimal Haemolysis units/MHU (the reciprocal of the highest dilution at which haemolysis was obtained) of each isolate was determined.

*Study on inactivation of L. monocytogenes during cooking of green leaves:* With the object of reproducing conditions of mallun preparation the time and temperature required for preparation of mallun was noted by heating fresh green leaves with coconut until they were partially cooked. Two samples of 250g chopped mukunuwanna (*Alternanthera sessilis*) which had been autoclaved were inoculated with a culture of *L. monocytogenes* ( $10^6$  organisms/g) and heated for 10 min to 82°C. Duplicate samples were removed for analysis of *L. monocytogenes* and temperature was recorded at 2 min intervals during the heating period of 10 min. The temperatures at 2 min intervals were 40, 53, 60, 72 and 82°C.

## RESULTS

### Isolation and characterization of *L. monocytogenes*

The initial identification of *L. monocytogenes* from food was based on the characteristic appearance of colonies on *Listeria* selective agar. The results of morphological and biochemical tests on food isolates were comparable with the reference strain *L. monocytogenes* (ATCC 19111).

### The occurrence of *L. monocytogenes* in food samples

*L. monocytogenes* was recovered from 52 samples out of 139 samples (38%) of food items tested (Table 1). The highest percentage of positives were observed in vegetables (49%), second in chicken (34%) and lowest in dairy products (26%).

**Table 1: Occurrence of *L. monocytogenes* in food.**

Type of Food	Number of samples examined	Number & Percentage of samples positive for <i>L. monocytogenes</i>
Raw chicken (Fresh & Frozen)	38	13 (34%)
Vegetables		
Green leaves	17	11 (65%)
Cabbage	18	6 (33%)
Lettuce	20	10 (50%)
Milk products		
Processed cheese (local)	2	0
(imported)	2	0
Cottage cheese (local)	1	0
Pasteurized milk	16	5 (31%)
Raw milk	12	3 (25%)
Ice cream (local)	12	4 (33%)
Fresh cream (local)	1	0
Total	139	52 (38%)

### Quantitation of haemolytic activity (Microhaemolysis test)

The minimal haemolysis units of isolates ranged from 16 to 64 with a mean of 38.85. The *L. monocytogenes* control strain (ATCC 19111) gave a MHU of 16.

### Inactivation of *L. monocytogenes* during cooking of green leaves

The actual temperature reached during cooking of green leaves into a mallun was about 69°C and the time of heating was 6 min. When the conditions of mallun preparation was reproduced with test samples of green leaves, *L. monocytogenes* was recovered from samples upto 8 min of cooking by which time the temperature was 72°C.

## DISCUSSION

This study is the first report of the isolation of *L. monocytogenes* from food in Sri Lanka. The haemolytic activity of *L. monocytogenes* is used in routine typing of *L. monocytogenes*. The haemolytic activity of *L. monocytogenes* local isolates (16-64 MHU) were within the range of reported minimum haemolytic activity (12-192 MHU) of *L. monocytogenes*.<sup>14</sup> The other species of *Listeria* which showed haemolytic activity were *L. seeligeri* and *L. ivanovii* with MHU of 3-6 and 384 respectively.<sup>14</sup>

The occurrence of *L. monocytogenes* in raw chicken has been widely studied. The reported contamination levels of raw chicken by *L. monocytogenes* in U.K.,<sup>39</sup> U.S.A.,<sup>15</sup> Denmark<sup>16</sup> and Switzerland<sup>17</sup> are 60%, 23.3%, 47.1% and 12% respectively. The contamination of poultry products, is due to listeriosis in poultry, asymptomatic healthy carriers<sup>16</sup> and contamination of carcasses via work surface and equipment.<sup>18,19</sup> A survey in U.K. showed that 12% of cooked ready-to-eat chicken too contained *L. monocytogenes*.<sup>20</sup> *L. monocytogenes* is not especially heat resistant and is killed at normal pasteurization temperature.<sup>21-23</sup> Therefore listeriosis due to cooked poultry<sup>24</sup> is linked either to inadequate cooking or recontamination after cooking. The results of the present study too indicates that 34.2% of raw chicken samples contained *L. monocytogenes*. However the Sri Lankan practice of thoroughly cooking chicken in the form of chicken curry probably reduces the risk of listeriosis through poultry meat. Nevertheless fast food outlets serving various other preparations of poultry meat may pose a threat as poultry meat may be inadequately cooked.

Raw vegetables are considered as an important vehicle of listeriosis. A large outbreak of listeriosis in 1981 was due to the use of contaminated cabbage in the preparation of coleslaw in Halifax, Canada.<sup>25</sup> Raw vegetables have caused outbreaks in Boston, U.S.A.<sup>8</sup> and in Australia.<sup>26</sup> A study on the occurrence of *L. monocytogenes* in raw retail vegetables in U.K. in 1987/1988 indicated that cabbage, cucumber, potatoes and radish were contaminated. The percentage of positive samples varied from 1.1 to 21.2.<sup>27</sup> In the present study of vegetables the isolation was highest from green leaves (gotukola-*Centella asiatica*, mukunuwanna-*Alternanthera sessilis*, kankun-*Ipomoea aquatica*, sarana-*Trianthema decandra*). *L. monocytogenes* can easily propagate in nature and can maintain a non-zoonotic life cycle in soil, water and vegetation.<sup>16,28</sup> It has also been shown that this organism does not lose virulence during long storage periods in soil.<sup>29</sup> Since green leaves are grown in low lying areas with plenty of water it is possible that these conditions facilitate contamination with *L. monocytogenes*.<sup>28</sup>

The practice of eating green leaves either raw or partially cooked as mallun is common in Sri Lanka. The present study indicated that *L. monocytogenes* was probably not inactivated during the preparation of a mallun. Cooking time and temperature should exceed 8 min and 72°C respectively in order to inactivate a heavy inoculum of *L. monocytogenes*. Lettuce and cabbage too were found to be contaminated and adequate precautions should be taken when preparing salads. It has been shown that when vegetables are held at 4°C for 4 d the

population of *L. monocytogenes* can increase twofold.<sup>30</sup> None of the vegetables used in the present study were refrigerated, but use of refrigeration as in supermarkets and homes increases the risk due to multiplication during refrigerated storage.

Presence of *L. monocytogenes* in raw milk has been reported in Netherlands (4.4%),<sup>31</sup> U.S.A. (4.5%),<sup>11</sup> Canada (1.3%),<sup>23</sup> Scotland (2.6%),<sup>32</sup> and Spain (45.3%).<sup>33</sup> It is eliminated by normal high temperature short time pasteurization<sup>34-36</sup> and its presence in pasteurized milk is either due to inadequate heating or post pasteurization contamination. Contamination of pasteurized milk in U.K. (1.1%)<sup>37</sup> and Northern Ireland (1.05%)<sup>38</sup> by *L. monocytogenes* has been reported. Presence of this organism in local pasteurized milk is highly significant. A study of soft cheese in U.K. showed the occurrences of *L. monocytogenes* in soft cheese manufactured in different countries were 14% in France, 4% in U.K., 16% in Italy and 10% in Cyprus.<sup>39</sup> The only local soft cheese available was cottage cheese and it did not contain *L. monocytogenes* in the present study.

Many outbreaks have been traced to dairy products such as pasteurized milk in Boston, U.S.A.,<sup>7</sup> soft cheese in California, U.S.A.<sup>40</sup> Therefore contaminated processed milk products as found in the present study pose the greatest threat from *L. monocytogenes* because they are consumed without further treatment.

In conclusion it can be said that those engaged in the food industry should take adequate measures to eliminate the organism during processing, avoid post processing contamination and check sample products for contamination.

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