Original article

Listeria monocytogenes and other Listeria species in retail meat and milk products in Addis Ababa, Ethiopia

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Abstract

Background: Listeriosis is one of the important emerging bacterial zoonotic infections worldwide. Among the different species of the genus Listeria, *Listeria monocytogens* is known to cause listeriosis in humans and animals. Information on the occurrence and distribution of *Listeria monocytogenes* and other Listeria species is very limited both in the veterinary and public health sectors in Ethiopia.

Objectives: The present study was undertaken to determine the occurrence and distribution of *Listeria monocytogenes* and other Listeria species in retail meat and milk products in Addis Ababa, Ethiopia.

Methods: A total of 316 food samples were collected using a cross-sectional study design from September 2003 to April 2004. The techniques recommended by the International standards Organization (ISO 11290-1, 1996) and the French Association for Standardization (AFNOR, 1993) were employed for the isolation and identification of Listeria species. Serotyping of *Listeria monocytogenes* was carried out at the French Authority for Food Safety (AFSSA), Ploufragan, France.

Results: Out of the total of 316 samples examined, 103 (32.6%) were found to be positive for Listeria. Listeria species were isolated in 69.8% (37/53), 47.5% (29/61), 43.5% (20/46), 18.6%, (8/43), 15.4% (8.52) and 1.6% (1/61) of the pork, minced beef, ice cream, fish, chicken and cottage cheese samples respectively. *Listeria monocytogenes* was detected in 5.1% of the samples analysed. It was isolated mainly from ice cream (19.6%) and pork samples (7.5%) followed by minced beef (1.6%), fish (2.3%) and chicken samples (1.9%). The serotypes of *Listeria monocytogenes* identified belonged to 1/2b, 4b and 4e. In addition to *Listeria* monocytogenes, other Listeria species identified were *Listeria (L). innocua* (65%), *L. seeligeri* (8.7%), *L. welshimeri* (6.8, *L.murrayi* (*L. ivanovii* and *L. grayi* (each 0.9%).

Conclusion: This study demonstrated the widespread occurrence and distribution of *L. monocytogenes* and other *Listeria* species in retail meat and milk products in Addis Ababa, Ethiopia. [*Ethiop.J.Health Dev.* 2004;18(3):208-212]

Introduction

Listeriosis is one of the important emerging bacterial zoonitic diseases that occur in humans a variety of animal humans. It arises mainly from the consumption of contaminated food products (1, 2). Reports indicate that listeriosis has emerged to be more important in developed countries but is reported less frequently in developing countries (3). This could be associated with lack of awareness of laboratory technicians or lack of diagnostic facilities and limited resources together with the presence of other disease epidemics that claim more priority than listeriosis in developing countries including Ethiopia.

Among the different species of the genus Listeria, *L. monocytogenes* has been known to cause listeriosis in humans and animals (4, 5). The other pathogenic species is *L. ivanovii*, which causes abortion in animals (4). *Listeria* species are ubiquitous in the environment and possess unique physiological characteristics that allow growth at refrigeration temperature that are usually adverse for most pathogenic, food-borne bacteria. The organism can also tolerate a pH between 5.4 and 9.6 (6). Numerous reports implicated food types such as milk and milk products, meat and meat products, raw vegetables and sea foods as sources of food borne listeriosis. Of particular concern are ready-to-eat foods that are refrigerated before consumption and those that do not undergo any substantial heat treatment (7-10).

Listeriosis is of major veterinary importance animals used for food particularly in cattle, sheep and goats. It causes encephalitis, abortion and septicemia (10, 11). Various studies have shown that people at greater risk are pregnant women and foetal children, alcoholics, drug abusers, patients with corticosteroid therapy, AIDS patients and the elderly (2,3,10). Infection acquired in early pregnancy may lead to abortion, still birth or premature delivery. When listeriosis is acquired late in pregnancy it can be transmitted transplacentally and lead to neonatal listeriosis (2, 10).

The available current literature shows that *L. monocytogenes* and other *Listeria* species have been reported from a wide variety of food types and clinical samples in various countries of the world (6, 7, 8, 9, 11, 12). Published information on the status of food borne listeriosis is very limited both in the veterinary and public health sectors in Ethiopia. This study reports the occurrence and distribution of *L. monocytogenes* and

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other L*isteria* species in retail meat products (minced beef, pork, chicken and fish), cottage cheese and ice cream purchased from supermarkets and other shops in Addis Ababa, Ethiopia.

Methods

Food samples: A cross-sectional study was undertaken to determine the prevalence and distribution of *Listeria monoctogenes* and other Listeria species from food samples. A total of 316 food samples consisting of minced beef (61), pork (35), chicken (52), fish (43), cottage cheese (61) and ice cream (46) were randomly selected and purchase from retail supermarkets and other shops in Addis Ababa from September 2003 to April 2004. The samples were kept in an icebox containing ice packs and immediately transported to the microbiology laboratory of the Faculty of Veterinary Medicine, at Debre Zeit town. They were processed upon arrival or stored at freezing temperature until analyzed. Frozen samples were thawed at room temperature four to six hours before processing.

Isolation and identification of Listeria species: For the isolation and identification of *Listeria* species in the food samples, the techniques recommended by the International Standards Organization (ISO 11290-1) (13) and the French Association for Standarization (AFNOR) (14) were employed. PALCAM (Polymixin Acriflavine Lithium chloride Ceftazidime Aesculin Mannitol) agar was need for selective plating and identification of Listeria colonies.

Primary selective enrichment: The primary selective enrichment step involved a selective liquid median with reduced concentrations of selective agents and the medium is known as half Fraser broth. The half Fraser broth enrichment medium (AES Lab., Combourg, France) contained one volume of lithium chloride (3g/10 mo of distilled water) and half a volume of both acriflavine hydrochloride (0.25 g/100 ml distilled water) and sodium salt of nalidixic acid (0.1 g/10 ml sodium hydroxide solution) (half Fraser broth). Twenty-five grams of each sample was added to a stomacher bag containing 225 ml of half Fraser broth. The mixture was homogenized using a laboratory blender (Stomacher 400, Seward, England) at high speed for 2 minutes. The test portion was incubated at 30^{o}C for 24 hours.

Secondary selective enrichment: The secondary selective enrichment medium (Fraser broth: AES Lab., Combourg, France) with full concentration of selective agents was employed. From the pre-enrichment culture (half Fraser broth), 0.1 ml was transferred into 10 ml of Fraser broth and incubated at 37^{o}C for 48 hours.

Isolation and identification: From the culture obtained in Fraser broth, a loopful of the culture was streaked onto PALCAM agar plates (AES Lab., Combourg, France) and incubated at 37^{o}C for 24 to 48 hours. The plates were examined for the presence of characteristic colonies presumed to be Listeria. Identification of Listeria species on PALCAM agar plates was based on aesculin hydrolysis and mannitol fermentation. All Listeria species hydrolyse aesculin as evidenced by a blackening of the medium. Mannitol fermentation was demonstrated by a colour change in the colony and/or surrounding medium from red or gray to yellow due to the production of acidic end products. The selectivity of the PALCAM medium is achieved through the presence of lithium chloride, polymixin B sulphate and acriflavine hydrochloride present in the medium base and ceftazidime provided by PALCAM antimicrobial supplement. These agents effectively suppress growth of most commonly occurring non-Listeria species of bacteria present in food samples.

Confirmation: Colonies suspected to be Listeria were transferred onto pre-dried plates of trytic soya years extract agar (TSYEA) (Difco, Bacton, USA) and incubated at 37^{o}C for 18 to 24 hours. Those putative Listeria colonies were characterized using Gram staining, motility and catalase test, characteristics of haemolysis, carbohydrate utilization and CAMP (Christe Atkins Munch Peterson) test following standard methods (ISO 11290-1, 1996; AFNOR, 1993).

The CAMP test was undertaken using *Staphylococcus aureus* (CIP: Collection of Institute of Pasteur, 5710) and *Rhodoccocus equi* (CIP 5869). They were streaked in single lines across a sheep blood agar plate so that the two cultures were parallel and diametrically opposite. Test strains were then streaked at right angles and 1 to 2 mm apart to *S. aureus* and *R. equi*. Simultaneously, control cultures of *L. monocytogenes*, *L. innocua* and *L. ivanovii* (kindly obtained from the French Authority for Food Safety (AFSSA), Ploufragan, France) were streaked onto blood agar plates. The plates were then incubated at 37^{o}C for 18 to 24 hours. An enhanced zone of beta hemolysis between the test strain and either of the culture of *S. aureus* and *R. equi* was considered a positive reaction (ISO 11290-1, 1996). *Listeria monocytogenes* showed an enhanced zone of hemolysis, forming an arrow head towards the *S. aureus* culture and *L. ivanovii* towards the *R. equi* culture while *L. innocua* is not hemolytic.

For the carbohydrate utilization test, isolated colonies from TSYEA were transferred into test tubes containing xylose, rhamnose and mannitol and incubated at 37^{0}C for up to 5 days. Positive reactions were indicated by yellow color (acid formation) and occured mostly within 24 to 48 hours. Serotyping of *L. monocytogenes* strains was carried out at the French Authority for Food Safety (AFSSA), Ploufragan, France.

Results

Out of a total of 316 food samples analysed, 103 (32.6%) were positive for Listeria. Listeria species were isolated

from minced beef, chicken, cheese, fish, ice cream and pork samples (Table 1). The level of contamination of food samples by Listeria species varied and was high in pork (69.8%), followed by minced beef (47.5%) and ice cream (43.5%).

Table 1: **Detection of Listeria species in different food types**

Sample type	Number of samples		%
	Examined	Positive	
Minced beef	61	29	47.5
Chicken	52	8	15.4
Fish	43	8	18.6
Pork	53	37	69.8
Cottage cheese	61	1	1.6
Ice cream	46	20	43.5
Total	316	103	32.6

The dominant Listeria species isolated in the present study was *L. innocua* (21.2%). It was frequently detected in pork (49.1%), followed by beef (34.5%) and chicken (13.5%) samples. *Listeria monocytogenes* was the second most frequently detected *Listeria* species (5.1%). Among the food samples tested, the contamination level by *L. monocytogenes* was high in ice cream (19.6%), followed by pork samples (7.5 %). *Listeria monocytogenes* was also isolated in 1.6 to 2.3% of other food samples (Table 2).

The isolated serotypes of *L. monocytogenes* belong to serotype 1/2b, 4b and 4e. Other *Listeria* species other than *L. monocytogenes, L. innocua* and *L. ivanovii* were also isolated from the different food samples analysed. These include *L. seeligeri, L. welshimeri, L. murrayi* and *L. grayi* (Table 2).

Table 2: **Distribution of Listeria species isolated from various types of food samples**

Listeria species	Number of Listeria species isolated from food samples						Total (%)
	Minced beef	Chicken	Cottage cheese	Fish	Ice cream	Pork	
L. monocytogenes	1	1	-	1	9	4	16 (15.5)
L. ivanovii	1	-	-	-	-	-	1 (0.9)
L. innocua	21	7	1	6	6	26	67 (65.0)
L. seeligeri	-	-	-	-	4	5	9 (8.7)
L. welshimeri	3	-	-	1	1	2	7 (6.8)
L. murrayi	2	-	-	-	-	-	2 (1.9)
L. grayi	1	-	-	-	-	-	1 (0.9)
Total	29 (12.8)	8 (7.7)	1 (0.9)	8 (7.7)	20 (19.4)	37 35.9)	103 (32.6)

Discussion

Listeriosis has been recognized to be one of the emerging zoonotic diseases during the last two decades and is contracted mainly from the consumption of contaminated foods and food products (9, 10, 15). Increasing evidence suggests that substantial portions of cases of human listeriosis are attributable to the food borne transmission of *L. monocytogenes* (10, 16). In our study, 32.6% of 316 food samples examined were positive for *Listeria* of which 5.1% were *L. mononcytogenes*. This was comparable with results of surveys undertaken in other countries (7, 9, 11, 12, 17). This suggests the presence of a significant public health hazard linked to the consumption of foods contaminated with *L. monocytogenes*.

Raw meat products, as expected showed a high level of contamination with Listeria species (50.6%). It is generally assumed that such products cannot be free from Listeria because of slaughter methods evisceration and food processing that allow greater chance for contamination. Furthermore, Listeria species are ubiquitous in the environment (18). People handling food at different levels can also be sources of contamination. In our study, a significant contamination level with Listeria species was observed in minced beef (47.5%). The majority of the species isolated in this food item were *L. innocua* comprising 34.4 % of the samples followed by *L. welshimeri* (4.9%) and *L. murrayi* (3.3%). The overall finding coincides with the report of other studies which indicated a 1 to 70% prevalence of *Listeria* species in beef samples (6, 11, 18). The tradition of consuming raw or undercooked meat exacerbates the public health risk associated with *L. monocytogenes*. Ryu and colleagues (19) reported the presence of *L. monocytogenes* in raw meat slices, ground meat and in meat processing environments. In addition, further processing and handling of meat and coating with spices increases the risk of contamination with *Listeria* species (8). Various authors have also indicated that food re-contamination might result from contact with equipment and mishandling of food products (2, 6, 10). *Listeria ivanovii* was isolated only from minced beef sample (1.6%). Though not common, *L. ivanovii* have been associated with cases of human listeriosis (6).

Of the 52 chicken samples examined, 15.4% contained different *Listeria* species comprising 1.9% *L. monocytogenes* and 13.5% *L. innocua.* Similarly, a high level of contamination of chicken meat with *Listeria* species has been reported elsewhere (7, 18). In our study, pork was found to be highly contaminated with *Listeria*

species (68.9%). *Listeria monocytogenes* was isolated from 7.5% of the pork samples, which was higher than those from other meat samples. Different authors reported 0 to 68% Listeria prevalence in pork samples (2, 6, 7, 8, 9, 11, 12). It has also been recognized that pork is most often exposed to contamination. This may be done to the feeding habit of pigs that may expose them to ingest Listeria contaminated feed in large quantities. It has also been reported that 45% of pigs harbored *L. monocytogenes* (6). Lanciotti and colleagues (20) also indicated that pork had a higher prevalence of *L. monocytogenes* (17.6%) among the meat samples examined including poultry and beef. Raw fish samples examined in this study showed 18.6% prevalence of Listeria species. However, *L. monocytogenes* was isolated from a single fish sample (2.3%). This complies with previous reports of 2.9% prevalence in raw fish (11). A research conducted in Japan showed a 6.1% prevalence of *L. monocytogenes* in fish and fish products (19).

An analysis of cottage cheese samples showed that 1.6% was contaminated with only *L. innocua*. The low contamination rate might be attributed to the fact that death of most of the organisms occurs in cottage cheese that tend to have a low pH and is also partly related to heat treatment. Our finding was in agreement with previous reports that indicated a prevalence rate of 0 to 10% depending upon the type of cheese and the production process (18, 20, 21).

Of the 46 ice cream samples examined, 20 (43.5%) were found to be contaminated with Listeria species. Ice cream samples were highly contaminated with *L. monocytogenes* (19.6%) followed by *L. innocua* (13%) and *L. seeligeri* (8.7%). Ice cream is produced from milk, butter, fruits, eggs and other additives. These components may provide adequate nutritional support for listerial growth and multiplication. Cordano and Rocourt (12) isolated *L. monocytogenes* from different ice cream flavors (vanilla and chocolate). The increased contamination might be due to the nature of ice cream that provides a suitable environment with respect to pH, water activity, nutrient availability and storage temperatures (22, 23). Isolation of *L. monocytogenes* from ice cream and frozen foods indicates the fact that it can withstand freezing temperature (16). Frequent occurrence of *L. monocytogenes* in ready-to-eat food items such as ice cream, which require no thermal treatment, implies increased public health risk associated with this pathogen.

In the present study, *L. innocua* and *L. monocytogenes* were more frequently isolated as compared with species of Listeria. *Listeria,, innocua* being the predominant isolate. Other studies also indicated that *L. innocua* is the most prevalent Listeria species found in food products (5, 20). Petersen and Madsen (24) found out that *L. innocua* grows faster than the pathogenic species in enrichment broth media and may, therefore, overgrow *L. monocytogenes* where both species are present in about the same numbers. Reports have indicated that *L. innocua* occupies the same ecological niche and its high incidence signifies potential contamination by *L. monocytogenes* (11, 20). The other nonpathogenic species of Listeria (*L. murrayi, L. seeligeri, L. welshimeri* and *L. grayi)* were detected to exist lower frequencies.

The serological analysis of *L. monocytogenes* strains showed that the serotypes which are usually involved in food-borne listeriosis outbreaks (1/2b and 4b) were also detected in the food samples analysed in this study. *Listeria monocytogenes* has thirteen serotypes, but, only three serotypes- 4b, 1/2a and 1/2b- are responsible for the majority of veterinary and human listeriosis cases (25). Serotype 4b has been identified as the cause of most human listeriosis cases whereas serotypes 4a and 4c are most of the time limited to animals (2, 25).

The expanding population of immuno-compromised people together with the high prevalence of the organism in foods indicates the necessity for establishing appropriate control and preventive measures to reduce risks associated with the occurrence of listeriosis (1, 18). Although pasteurization of milk efficiently kills *L. monocytogenes*, post-pasteurization contamination of dairy products is still high. Regarding meat and meat products, post-processing manipulations appear to be the means by which the products are re-contaminated (3, 20). The risk of cross-contamination from raw to cooked foods during storage and preparation necessitates a control program for *L. monocytogenes* in addition to strict hygienic processing of foods. This helps to prevent the organism from entering the food chain (6, 7, 10).

In conclusion, This study has demonstrated the presence and distribution of *L. monocytogenes* and other Listeria species in a variety of raw and ready-to-eat food products in Addis Ababa. The study also suggests the need for improved food safety through the implementation of hygienic measures at all levels from production to consumption with particular emphasis on ready-to-eat food items which require no further heat treatment.

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