View metadata, citation and similar papers at core.ac.uk



http://www.elsevier.com/locate/jiph

Isolation and characterization of *Listeria monocytogenes* and other *Listeria* species in foods of animal origin in Addis Ababa, Ethiopia

Simon Gebretsadik^a, Tesfu Kassa^b, Haile Alemayehu^b, Kahsay Huruy^a, Nigatu Kebede^{b,*}

^a Medical Faculty, University of Gondar, Gondar, Ethiopia ^b Aklilu Lemma Institute of Pathobiology, College of Health Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

Received 26 September 2009; received in revised form 28 September 2010; accepted 15 October 2010

KEYWORDS

Listeria monocytogenes; Listeriosis; Public health; Veterinary; Foods of animal origin

Abstract Listeriosis is a disease of humans and animals, in which it is one of the important emerging bacterial zoonotic diseases worldwide. Among the different species of the genus Listeria, Listeria monocytogenes (L. monocytogenes) is known to cause listeriosis in humans and animals with low incidence but high case fatality rate. Information on the occurrence and distribution of L. monocytogenes and other Listeria species is very limited both in the veterinary and public health sectors in Ethiopia. The objective of this study was to isolate and characterize L. monocytogenes and other Listeria species from foods of animal origin (cottage cheese, raw beef, raw milk and liquid whole egg) in Addis Ababa, Ethiopia. A total of 391 food samples of animal origin were collected randomly, using a cross-sectional study design from November 2008 to March 2009. L. monocytogenes isolation and characterization were performed according to mainly the United States Food and Drug Administration procedures. Of the samples examined, 102 (26.1%) were found to be positive for *Listeria*. *Listeria* species were isolated in 39 (51.3%), 37 (32.2%), 22 (22%) and 4 (4%) of the raw beef, liquid whole egg, raw milk and cottage cheese samples respectively. L. monocytogenes was detected in 5.4% of the samples analyzed. It was isolated mainly from raw milk (13%) and liquid whole egg (4.3%) followed by raw beef (2.6%) and cottage cheese (1%). In addition to L. monocytogenes, other Listeria species were identified as L. innocua (60.8%), L. welshimeri (6.9%), L. seeligeri (3.9%), L. murrayi (2.9%) and L. grayi (2.9%) and L. ivanovii (1.9%). It was shown that L. monocytogenes and other Listeria species are widely spread in occurrence in foods of animal origin in Addis Ababa, Ethiopia. © 2010 King Saud Bin Abdulaziz University for Health Sciences. Published by Elsevier

© 2010 King Saud Bin Abdulaziz University for Health Sciences. Published Ltd. All rights reserved.

* Corresponding author. Tel.: +251 11 2763091; fax: +251 11 2755296. *E-mail addresses*: nigatukebede@yahoo.com, knigatu2003@yahoo.com (N. Kebede).

1876-0341/\$ - see front matter © 2010 King Saud Bin Abdulaziz University for Health Sciences. Published by Elsevier Ltd. All rights reserved. doi:10.1016/j.jiph.2010.10.002

1. Introduction

The bacterium Listeria monocytogenes (L. monocvtogenes) and the disease listeriosis were first recognized in laboratory animals in Cambridge in 1924 [1]. It later became apparent that the disease also affects humans, and the rise during the 1980s in the number of human cases in several countries together with evidence for food borne transmission has seen much renewed interest in this disease [2,3]. This interest has resulted in great advances in the understanding of the distribution of the bacterium together with vastly improved methods for detection. In addition, there has also been a vast improvement to the understanding of the overall morbidity, mortality and epidemiology of the disease, together with the pathogenicity and disease mechanisms used by L. monocytogenes. However despite these advances, there continues to be public health problems concerned with the control of this bacterium, particularly with respect to the contamination of food [4].

The genus Listeria includes seven species, and these comprise L. monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. Ivanovii L. murrayi and L. grayi [5]. All members of the genus Listeria are widely distributed in nature and have been isolated from soil, vegetation, sewage, water, and animal feed, fresh and frozen meat including poultry, slaughter house wastes and in the faeces of healthy animals including humans. These organisms can become endemic in food processing environments [4]. L. monocytogenes is an intracellular Grampositive non-sporulating pathogenic bacterium with wide spread presence in nature, affecting a wide range of domestic and wild animals and humans [6]. In the vast majority of human cases, infection is the result of consumption of contaminated food [7]. Although the infectious dose remains unknown and is most likely host dependent, the resulting invasive disease, listeriosis, is a serious illness with a high fatality rate [8]. Owing to its complex and versatile physiological adaptation mechanisms, L. monocytogenes can persist and often proliferate in contaminated foods under a wide range of environmental conditions, such as-low water activity, low pH, and low temperature [9].

Human disease due to *L. monocytogenes* generally occurs in the setting of pregnancy or of immunosuppression caused by illness or medication. Increasing evidence suggests that a substantial portion of cases of human listeriosis are attributable to the food borne transmission of *L. monocytogenes*. Unlike most food borne pathogens, which cause primarily gastrointestinal illness, *L. monocytogenes* causes invasive syndromes, such as

meningitis, sepsis, chorioamnionitis, and stillbirth. In addition to human infection, *L. monocytogenes* long recognized as a veterinary pathogen, which causes basilar meningitis (''circling disease'') and stillbirth in sheep and cattle. The occurrence of listeriosis among humans has received increasing attention as the role of contaminated foods in the pathogenesis of epidemic listeriosis has been recognized and reports of disease associated with the expanding immunosuppressed population have accumulated [10].

This bacterium principally causes intra-uterine infection, meningitis and septicemia. Listeriosis in pregnancy manifests as severe systemic infection in the unborn or newly delivered infant as well as a mild influenza-like bacteraemic illness in the pregnant woman. Infection can occur at any stage of pregnancy. Listeriosis not associated with pregnancy usually affects persons with immunosuppressive conditions, although invasive disease can also affect immunocompetent adults, particularly elderly persons. Listeriosis in children older than 1 month is very rare, except in those with underlying disease. In adults and juveniles, the main presentations are as central nervous system infection and/or septicemia [11]. The mortality rate in systemic listeriosis has been estimated between 20% and 40% [12], and survivors, particularly those where the organism has invaded the central nervous system, can develop serious long-term sequel. In England and Wales, an incidence of 1.7-2.4 cases per million between 1995 and 1999 was estimated [13]: this compares with 5.4 and 9.4 cases per million in France and the USA, respectively [14,15].

Meat, poultry and dairy products have been all identified as vehicles of contamination for listeriosis in humans [16,17]. The widespread incidence of Listeria species in raw milk was reported in several states of America, Canada and in many countries of Europe [18]. High incidence and isolation of *Lis*teria species from food and environmental samples including raw beef in Enugu State of Nigeria was also reported [19]. More specific work related to dairy products showed rather low level of raw milk (only 3-4%) and post pasteurization contamination from environment sources [20]. In sum, the available literature shows that L. monocytogenes has been reported from a wide variety of food types and responsible for outbreaks and clinical manifestations in various countries of the world [21]. Although foods of animal origin such as milk, cheese, meat and poultry are consumed well in Ethiopia, published information on the status of food borne listeriosis caused by L. monocytogenes, is very limited both in the veterinary and public health sectors. In addition, the occurrence of the bacteria in foods of animal origin is not yet well documented [22]. Therefore, the aim of this study is to isolate and characterize *L. monocytogenes* and other *Listeria* species in foods of animal origin.

2. Materials and methods

A cross-sectional study was undertaken to determine the prevalence and distribution of *L*. *monocytogenes* and other *Listeria* species from foods of animal origin. In the study for isolation and identification of *Listeria* species in the food samples, the procedures described by the United States Food and Drug Administration and Center for Food Safety and Applied Nutrition [23] and Curtis and Lee [24] were employed. For the best possible recovery of *Listeria* species, pre-enrichment, selective enrichment and selective plating, and the recommended and alternative procedures were followed.

2.1. Food samples

A total of 391 food samples consisting of 115 liquid whole eggs, 76 raw beef, 100 raw milk and 100 cottage cheese samples were purchased randomly from municipally licensed retail shops, butchers, cafeterias and markets of different sub cities of Addis Ababa respectively from November 2008 to March 2009. The samples were kept in an icebox containing ice packs and immediately transported to the food Microbiology laboratory of Aklilu Lemma Institute of Pathobiology at Addis Ababa. They were processed upon arrival or stored at freezing temperature until analyzed. Frozen samples were thawed at room temperature 4–6 h before processing.

2.2. Sample preparation and inoculation

Twenty-five grams of raw beef sample was chopped randomly from each of the purchased 500 g beef samples using sterile knife; this was transferred in to sterile plastic bag which contained 225 ml of buffered *Listeria* pre-enrichment broth (tryptone soya broth, Oxoid Ltd., Hampshire, UK) and homogenized using a laboratory blender (Stomacher 400, Seward, England) at high speed for 2 min. Finally the whole sample was transferred to sterile bottle and was kept inside the incubator at 30 °C for 48 h.

The other food samples (egg, cheese and milk) were mixed thoroughly by taking 25g of each, in the following manner: each of the 25g of whole liquid egg and raw milk was weighed in sterile beakers separately; similarly the 25g of cheese was weighed

after homogenizing thoroughly using sterile spoon. For the latter samples, the rest of the procedure followed was the same as indicated for the raw beef. One ml of the suspension was drawn and inoculated in 9 ml of UVM I (University of Vermont Medium, Oxoid Ltd., Hampshire, UK) and incubated for 24 h at 35 °C. Then, well mixed 1 ml of UVM I suspension was transferred to 9 ml of UVM II (Oxoid Ltd., Hampshire, UK) and incubated at 35°C for 24 h. The UVM I was used as the primary Listeria selective enrichment medium and UVM II as the secondary enrichment; the difference between UVM broths I and II is simply the inclusion of a higher concentration of acriflavine in the latter [24]. The reduced amount of the selective agents contained in the primary selective enrichment medium allows resuscitation of sublethally injured cells.

2.3. Isolation and identification

PALCAM (Polymyxin Acriflavin Lithium Chloride Ceftazidime Aesculin Mannitol) agar (Merck, Darmstadt, Germany) is used for selective plating and identification of Listeria colonies [25,26,22]. As a continuation of the procedure from the selective enrichment broth II (UVM II, Oxoid Ltd., Hampshire, UK) in Section 2.2, a loop-full of suspension was taken and inoculated in to the selective media, PALCAM agar and the plate was incubated at 35 °C for 24-48 h. Growth of 1-2 mm diameter black or black green colony with a black halo and black sunken center was taken as positive for Listeria species [23]. When the colonies were grown and well isolated on selective media, 5 colonies from each medium were picked and suspended in to tryptose soya broth yeast extract (TSBYE, Oxoid Ltd., Hampshire, UK). It was incubated at 37 °C for about 1 h till slight turbidity was visible and the suspension was inoculated onto plates of tryptic soya veast extract agar (TSYEA, Difco, Becton, USA) to obtain pure culture for biochemical confirmation and incubated for 24h at 37°C. The above steps and use of several media was shown to give substantially improved isolation, with purity and typical isolated Listeria colonies; purification on TSYEA is considered as a mandatory step in the conventional analysis [23,27].

Colonies suspected to be *Listeria* were confirmed by different species specific tests, they were transferred onto TSYEA, in a manner to allow well-separated colonies to develop and that was incubated at 37 °C for 18–24h. Those pure *Listeria* colonies were characterized using Gram staining, motility and Catalase test, characteristics of haemolysis, carbohydrate utilization and CAMP (Christie–Atkins–Munch–Peterson,

Species	Acid produc	ced from	Hemolysis	CAMP test	
	Xylose	Rhamnose	Mannitol		S. aureus
L. monocytogenes	_	+	+	+	+
L. innocua	_	V	_	_	_
L. ivanovii	+	_	_	++	_
L. seeligeri	+	_	_	(+)	(+)
L. welshimeri	+	V	_	_	_
L. grayi	_	_	+	_	_
L. murrayi	_	V	+	_	_

Table 1 Biochemical differentiation and characteristics of Listeria species.

+: >90% positive reaction; (+): weak reaction; V: variable; -: no reaction.

Biomerieux, France) test following standard methods (USFDA/CFSAN, Bacteriological Analytical Manual [23]. All Listeria species are Gram-positive rods, catalase-positive and are slim, short rods with slight rotating motility. The CAMP test is a useful tool to identify the species of a Listeria species isolate; the test was undertaken using standard strain of *Staphylococcus aureus* (S. *aureus*) (American Type Culture Collection (ATCC) 25923) by streaking vertically on sheep blood agar and streaking test organisms (Listeria isolates) horizontally (perpendicular) to S. aureus streak [22]. Simultaneously, control cultures of L. monocytogenes (ATCC 35152 and ATCC 19116), L. innocua (ATCC 33090) and L. ivanovii (ATCC 19119) (kindly obtained from the Ethiopian Health and Nutrition Research Institute (EHNRI) and National Animal Health Diagnostic and Investigation Center (NAHDIC), Addis Ababa, Ethiopia) were streaked onto sheep blood agar plates (Oxoid Ltd., Hampshire, UK). The plates were then incubated at 37°C for 18-24h. Test results were read and an enhanced arrow head zone of beta hemolysis between the test strain and the culture of S. aureus was considered a positive reaction (Table 1). L. monocytogenes showed an enhanced zone of hemolysis, forming an arrow head towards the S. aureus culture and L. seeligeri shows weak hemolysis while other Listeria species are not hemolytic [23]. For the carbohydrate utilization test, isolated colonies from TSBYE were transferred into test tubes containing xylose, rhamnose and mannitol (Scharlau, Barcelona, Spain) and incubated at 37 °C for up to 7 days. Positive reactions were indicated by yellow color (acid formation) and occurred mostly within 24–48 h. Table 1 presents the biochemical differentiation and characteristics of Listeria species.

Quality control: The correct performance of all stages of the analysis, including enrichment, screening tests, plating and all confirmatory tests were verified through the use of appropriate controls.

2.4. Statistical analysis

The collected data were treated using descriptive analysis. The contingency table analysis was based on the chi square test (χ^2 Pearson). Fisher's exact test was used as appropriate.

3. Results

A total of 391 food samples of animal origin purchased from 8 sub-cities of Addis Ababa were analyzed for possible contamination with *Listeria*. On the basis of *Listeria* colonies isolated using PAL-CAM agar plates, a total of 102 (26.1%) food samples were positive for *Listeria*. The detection of *Listeria* species contamination in the different food types (cottage cheese, raw beef, raw milk and liquid whole egg) is shown in Table 2. Contamination by *Listeria* species was found significantly high in raw beef 39 (51.3%), followed by liquid whole egg 37 (32.2%) and raw milk 22 (22%) ($\chi^2 = 53.4643$, P = 0.000).

Using biochemical tests and the characteristics of *Listeria* colonies, the isolates were differentiated to species level (Table 1). The distribution of *Listeria* species isolated from different types of

Table 2Detectionfood items of anima		
Sample type	No. of samples examined for <i>Listeria</i> spp.	No. (%) of samples positive ^a for <i>Listeria</i> spp.
Cottage cheese	100	4 (4.0)
Raw beef	76	39 (51.3)
Raw milk	100	22 (22.0)
Liquid whole egg	115	37 (32.2)
Total	391	102 (26.1)
^a $\chi^2 = 53.4643, P = 0$.000.	

25

Listeria species	Number of Listeria species isolated							
	Cottage cheese	Raw beef	Raw milk	Liquid whole egg	Total (%)			
L. monocytogenes	1	2	13	5	21 (20.6) ^a			
L. innocua	1	30	4	27	62 (60.8) ^b			
L. ivanovii	0	1	0	1	2 (1.9)			
L. seeligeri	0	0	3	1	4 (3.9)			
L. welshimeri	1	5	0	1	7 (6.9)			
L. grayi	0	0	1	2	3 (2.9)			
L. murrayi	1	1	1	0	3 (2.9)			
Total	4 (3.9)	39 (38.2)	22 (21.6)	37 (36.3)	102 (26.1)			

Table 3 Distribution of Listeria species isolated from different types of food samples of animal origin.

^a $\chi^2 = 15.0867$, P = 0.002.

^b $\chi^2 = 7.6999, P = 0.053.$

food samples is presented in Table 3. The dominant *Listeria* species isolated in the present study was *L.* innocua 62 (15.9%) ($\chi^2 = 7.6999$, P = 0.053). It was frequently detected in raw beef 30 (39.5%), followed by liquid whole egg 27 (23.5%) and raw milk 4 (4%) samples. *L. monocytogenes* was the second most frequently isolated *Listeria* species 21 (5.4%). Among the food samples analyzed, the prevalence of *L. monocytogenes* was the highest in raw milk 13 (13%), followed by liquid whole egg 5 (4.3%), raw beef 2 (2.6%) and cottage cheese 1 (1%) food samples ($\chi^2 = 15.0867$, P = 0.002; Table 3).

Listeria species other than L. monocytogenes and L. innocua were also isolated from the different food samples analyzed. These include L. welshimeri 7 (1.8%), L. seeligeri 4 (1%), L. grayi 3 (0.8%), L. murrayi 3 (0.8%) and L. ivanovii 2 (0.5%) (Table 3). The isolation of Listeria species was made for different food items collected from various sites. The prevalence of the specific species in different sub-cities of Addis Ababa is shown in Table 4. The highest number of L. monocytogenes contaminated samples was from Yeka 4 (19%) ($\chi^2 = 1.6245$, P = 0.99) and the highest number of *Listeria* species was recorded in Arada sub-city ($\chi^2 = 0.6035$, P = 0.999).

4. Discussion

Listeriosis has been recognized to be one of the emerging zoonotic diseases during the last decades contracted mainly from the consumption of contaminated foods and food products. Increasing evidence suggests that substantial portions of cases of human listeriosis are attributable to the food borne transmission of *L. monocytogenes* [22]. *L. monocytogenes* continues to be of interest to the food industries and regulating agencies worldwide, to the scientists in various disciplines, and to the consumers of ready to eat foods. Sporadic cases of listeriosis continue to occur worldwide and there have been several outbreaks of the disease associated with food in USA and Europe. The findings

Site of collection (sub-cities)	Different types of Listeria species ^a								
	L.m	L.in	L.iv	L.s	L.w	L.g	L.mu	Unspecified	Total
Addis Ketema	3	8	1	0	1	0	0	0	13
Lideta	2	7	0	0	1	1	1	0	12
Lafto	1	8	0	1	1	0	0	0	11
Kirkos	3	7	0	0	0	0	1	1	12
Bole	3	6	0	0	1	2	0	0	12
Arada ^b	2	7	1	2	2	0	1	0	15
Yeka ^c	4	10	0	0	0	0	0	0	14
Gulele	3	9	0	1	1	0	0	0	14
Total	21	62	2	4	7	3	3	1	103

Table 4 The distribution of <i>Listeria</i> species isolated from different s	ites of collection in Addis Ababa.
--	------------------------------------

^a L.m, Listeria monocytogenes; L.in, Listeria innocua; L.iv, Listeria ivanovii; L.s, Listeria seeligeri; L.w, Listeria welshimeri; L.g, Listeria grayi; L.mu, Listeria murrayi.

^b $\chi^2 = 1.6245, P = 0.99.$

 $^{c}\chi^{2} = 0.6035, P = 0.999.$

of the present study also showed that *L. monocytogenes* and other *Listeria* species are quite prevalent in different food items in Ethiopia. Similar conditions may also be prevailing in other developing countries, so the present study may initiate interest of Veterinarians, Medical persons and other agencies involved in food hygiene or food microbiology to the problem of listeriosis.

In this study, cottage cheese, raw beef, raw milk and liquid whole egg samples were chosen and analyzed for the presence of *L. monocytogenes* and other *Listeria* species. The predominant bacterium isolated was *L. innocua* 62 (15.9%). It is the most common isolate in Ethiopia as reported by other researchers [22]. It was also known to be the highest prevalent *Listeria* species elsewhere in the world [28,29]. *L. monocytogenes*, which was ranked in the second position, was found in 21 samples.

The first study on listeriosis in Ethiopia was done by Molla et al. [22], where they reported a prevalence of 32.6% *Listeria* species in all food samples. This study confirms the presence of *Listeria* species including *L. monocytogenes* in different foods of animal origin. Taking habit of eating raw food, the chance of encountering the disease is very high. In the present study *L. monocytogenes* was isolated in 5.4% and this is similar to the previous work. Research results from China have reported that, *L. monocytogenes* of 5.79%, from China food products [30].

The distribution of *Listeria* species in each food item is quantified in the following manner. About 100 cottage cheese samples were examined and four different types of *Listeria* species with total number of 4 (4%) were isolated. These were; *L. monocytogenes*, *L. innocua*, *L. welshimeri* and *L .murrayi* each of 1 (1%) was isolated. The overall finding is almost comparable with Molla et al. [22] report; it also coincides with most surveys, which suggests that 1-10% of cheeses were contaminated with *L. monocytogenes* and other *Listeria* species [31].

In this study, raw beef was found to be the main source of *Listeria* species, including *L. monocytogenes*. Out of 76 raw beef samples examined, more than half (39/76) were contaminated with *Listeria* species, in which 2/21 *L. monocytogenes* were detected. All *Listeria* species except *L. seeligeri* and *L. grayi* were identified. It could be that the beef samples were exposed to poor sanitary conditions, during slaughtering, processing and selling. This finding is in agreement with earlier report [22] and other studies [32] that isolated *Listeria* species from raw meat as high as 50%. Where as in the case of raw milk, *Listeria* species were isolated with the total number of 22 (22%). *L. monocytogenes* was the most isolated species 13 (59.1%). Even if there is no report on prevalence of L. monocytogenes in raw milk in our country, this study showed very high prevalence rate comparing with other countries [20,33]. This high isolation rate might be due to difference in the site of collection of milk in this study (raw milk sample was collected from cafeterias, in which it is believed that milk is diluted with water before selling to the consumers) and in others, the difference in hygiene, environmental contamination rates and also poor milking practices [34]. For instance, wash water used on farm conditions can serve as a source of microbial contamination of soiled equipment and/or the milk [35]. In addition to L. monocytogenes other Listeria species were also isolated; these are L. innocua 4 (4%), L. seeligeri 3 (3%), L. gravi and L. murravi each of 1 (1%). Therefore, there are many chances of contamination, when it is diluted with water, from handlers or environment and milkers.

In this study, about 115 liquid whole egg samples were tested and six types of Listeria species with a total number of 37 (32.2%) isolates were found; five of the isolates (4.3%) belonged to L. monocytogenes and is in agreement with a study conducted in Turkey [36]. In the latter work L. monocytogenes was isolated from 5 out of 123 (4.06%) randomly sampled liquid whole quail eggs. Although L. monocytogenes has been isolated on eggshells and in the environment of laying hens, in view of world outbreaks, there have been no documented human cases of food borne L. monocytogenes due to the consumption of eggs or egg products [21]; a more recent study reported 196 L. monocytogenes isolates and demonstrated 3 genotypes out of total of 144 liquid whole egg samples [37]. However, there was no report on the isolation of L. monocytogenes and other Listeria species in liquid whole egg in the country. This is the first of its kind to be reported in Ethiopia.

On the other hand, the isolate, which grew and showed similar growth characteristics on the selective media (PALCAM), like other Listeria species, but did not coincide with the actual biochemical characteristics at the species level, was termed as 'difficult to specify'. Because, it is difficult to include the isolate in the report, since its culture morphological characteristics looks like the Listeria species, but failed to meet any of the known species by biochemical tests. The food samples were collected from randomly chosen 8 sub-cities of Addis Ababa, the capital city of Ethiopia. L. monocytogenes was isolated in all of the sub-cities. These are Addis Ketema, Lideta, Lafto, Kirkos, Bole, Arada, Yeka and Gulele. The highest number of L. monocytogenes contaminated samples was obtained from Yeka 4 (19%), though statistically not significant ($\chi^2 = 1.6245$, P = 0.99). Addis Ketema, Kirkos, Bole and Gulele sub-cities rank second to the former place where three isolates were obtained in each. On the other hand, the number *L. monocytogenes* contaminated samples from Arada and Lideta were two in each sub-city, and one from Lafto. This study was time and resources bound, therefore much conclusion cannot be made from this work except *L. monocytogenes* is present in all sub-cities and dif-

ferent types of foods of animal origin.

In general, *Listeria* has been isolated from all 8 sub-cities of Addis Ababa and the highest number of *Listeria* species contaminated samples was found from Arada followed by Yeka and Gulele subcity, although that was not statistically significant. Based on this study, it was shown that all types of *Listeria* species are prevalent in Addis Ababa. In sum, the main objective of this study was to know the distribution of *L. monocytogenes* and other *Listeria* species, and it was proved to be prevalent in those indicated areas. The findings were similar to the previous work [22]. It could be safely said that these findings are almost similar in detection of *L. monocytogenes*, even though the examined samples were somewhat different.

In conclusion, this study has demonstrated the presence and distribution of L. monocytogenes and other Listeria species in different types of food of animal origin in Addis Ababa. From this understanding and the peculiar characteristics of listeriosis (high case fatality rate, emerging, opportunistic, and sporadic and cause death in immunocompromised individuals), the bacteria can pose a threat to human life and can cause similar outbreaks of morbidity and mortality here too. It is likely that people who have been suffering from unknown causes of meningitis, abortion, still birth, septicaemia, and so on, might be infected by L. monocytogenes. Therefore, creating public awareness by disseminating the information is necessary. In addition, serious precautions regarding the food type, storage system and proper cooking should be considered while handling of food, in order to control listerial food contamination. Moreover, conducting similar studies in other towns and in animal feed in the country by the use of serotyping or PCR techniques are recommended.

Funding

No funding sources.

Competing interests None declared.

Ethical approval Not required.

Acknowledgements

The authors would like to acknowledge Dr. Alehgn Wubete of the National Animal Health Diagnostic and Investigation Center and Miss. Muluwork Getahun and Miss. Frehiwot Abera of the Ethiopian Health and Nutrition Research Institute for providing us control strains (S. aureus, L. monocytogenes, L. innocua and L. ivanovii) and chemicals. Financial support was obtained from the Office of Vice President for Research and Graduate Studies of the Addis Ababa University, Ethiopia.

References

- Murray EGD, Webb RA, Swann MBR. A disease of rabbits characterized by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.). J Pathol Bacteriol 1926;29:407–39.
- [2] McLauchlin J. The relationship between *Listeria* and listeriosis. Food Control 1996;7:187–93.
- [3] McLauchlin J. The role of the PHLS in the investigation of listeriosis during the 1980s and 1990s. Food Control 1996;7:235-9.
- [4] McLauchlin J, Mitchell RT, Smerdon WJ, Jewell K. Listeria monocytogenes and listeriosis: a review of hazard characterization for use in microbiological risk assessment of foods. Int J Food Micrbiol 2004;92:15–33.
- [5] McLauchlin J, Jones D. Erysipelothrix and Listeria. In: Borriello SP, Duerden BI, editors. Topley and Wilson's microbiology and microbial infections. 9th ed. Systematic Bacteriology, CD Rom London, Arnold, vol. 2 Update 1; 1999.
- [6] Dowe MJ, Jackson ED, Mori JG, Bell CR. Listeria monocytogenes survival in soil and incidence in agricultural soils. J Food Prot 1997;60:1201–7.
- [7] Fenlon DR, Wilson J, Donachie W. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. J Appl Bacteriol 1996;81:641–50.
- [8] Fenlon DR. Wild birds and silage as reservoirs of *Listeria* in the Agricultural environment. J Appl Bacteriol 1985;59:537–43.
- [9] Charpentier E, Courvalin P. Antibiotic resistance in *Listeria* spp. Antimicrob Agents Chemother 1999;43:2103–8.
- [10] Aureli P. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. N Engl J Med 2001;342:1236–2000.
- [11] Bortolussi R, Schlech WF. Listeriosis. In: Remington JO, Klein JS, editors. Infectious diseases of the fetus and newborn infant. 4th ed. Philadelphia: Saunders; 1995. p. 1055–73.
- [12] Farber JM, Peterkin PI. Listeria monocytogenes, a foodborne pathogen. Microbiol Rev 1991;55:752–811.
- [13] Smerdon WJ, Jones R, McLauchlin J, Reacher M. Surveillance of listeriosis in England and Wales 1995–1999. Commun Dis Public Health 2001;4:188–93.
- [14] Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis 1999;5:607–25.
- [15] Goulet V, de Valk H, Pierre O, Stainer F, Rocourt J, Valliant V, et al. Effect of prevention measures on incidence of human listeriosis, France, 1987–1997. Emerg Infect Dis 2001;7:983–9.

- [16] Schlech 3rd WF, Lavigne PM, Bortolussi RA, Allen AC, Haldane EV, Wort AJ, et al. Epidemic listeriosis—evidence for transmission by food. N Engl J Med 1983;318: 203–6.
- [17] Jemmi T, Stephan R. Listeria monocytogenes: food-borne pathogen and hygiene indicator. Rev—Off Int Epizoot 2006;25:571—80.
- [18] Ryser ET, Marth EH. Listeria, *Listerosis*, and food safety. New York: Marcel Dekker In.; 1991.
- [19] Ikeh1 MAC, Obi1 SKC, Ezeasor DN, Ezeonu1 IM, Moneke AN. Incidence and pathogenicity profile of *Listeria* sp. isolated from food and environmental samples in Nsukka, Nigeria. Afr J Biotechnol 2010;9:4776–82.
- [20] Kozak J, Balmer T, Byrne R, Fisher K. Prevalence of *Listeria monocytogenes* in foods: incidence in dairy products. Food Control 1996;7:215–21.
- [21] Swaminathan B, Gerner-Smidt P. The epidemiology of human listeriosis. Microbes Infect (Institut Pasteur) 2007;9:1236–43.
- [22] Molla B, Yilma R, Alemayehu D. Listeria monocytogenes and other Listeria species in retail meat and milk products in Addis Ababa, Ethiopia. Ethiop J Health Dev 2004;18:131–212.
- [23] USFDA/CFSAN. Detection and Enumeration of L. monocytogenes in foods. Bacteriological Analytical Manual (BAM); January 2003, http://www.cfsan.fda.gov [Chapter 10].
- [24] Curtis GDW, Lee WH. Culture media and methods for isolation of *Listeria monocytogenes* in foods. Int J Food Microbiol 1995;26:1–13.
- [25] Capita R, Alonso-Calleja C, Prieto M, Fernandez MC, Moreno B. Comparison of PALCAM and modified Oxford plating media for isolation of Listeria species in poultry meat following UVMII or Fraser secondary enrichment broths. Food Microbiol 2001;18:555–63. Available online at http://www.idealibrary.com.
- [26] Scotter SL, Langton S, Lombard B, Schultend S, Nagelkerk N, In't Veld PH, et al. Validation of ISO method 11290 Part 1—Detection of *Listeria monocytogenes* in foods. Int J Food Microbiol 2001;64:295–306.

- [27] Warburton DW, Farber JM, Armstrong A, Caldeira R, Hunt T, Messier S, et al. A comparative study of the "FDA" and "USDA" methods for the detection of *Listeria monocytogenes* in food. Int J Food Microbiol 1991;13:105–18.
- [28] Safdar A, Armstrong D. Antimicrobial activities against 84 Listeria monocytogenes isolates from patients with systemic listeriosis at a comprehensive cancer center (1955–1997). J Clin Microbiol 2003;41:483–5.
- [29] Opinions of the scientific committee on veterinary measures relating to Public Health on Listeria monocytogenes. European commission, scientific health opinions; 1999. http://europa.eu.int.
- [30] Chao G, Zhou X, Jiao X, Qian X, Xu L. Prevalence and antimicrobial resistance of food borne pathogens isolated from food products in China. Foodborne Pathog Dis 2007;4:277–84.
- [31] Lovett J, Francis DW, Hunt JM. Listeria monocytogenes in raw milk. Detection, incidence and pathogenicity. J Food Prot 1987;50:188–92.
- [32] Rocourt J, Cossart P. Listeria monocytogenes. In: Doyle MP, Beuchat LR, Montiville TJ, editors. Food microbiology fundamentals and frontiers. Washington, DC: ASM Press; 1997. p. 337–51.
- [33] Chapman B. RTI—issues regarding raw milk sales and consumption, in session summaries, Members of the IAFP Student professional development group. Food Prot Trends 2006:824–45.
- [34] Peeler JT, Kelly BV. Hazard assessment of *L. monocy-togenes* in the processing of Bovine milk. J Food Prot 1994;57:689–97.
- [35] Bramley AJ, McKinnon CH. The microbiology of raw milk. In: Robinson RK, editor. Dairy Microbiology, I. London, NY: Elsevier Applied Sci; 1990. p. 171.
- [36] Erdoúrul Ö. Listeria monocytogenes, Yersinia enterocolitica and Salmonella enteritidis in Quail Eggs. Turk J Vet Anim Sci 2004;28:597–601.
- [37] Rivoal K, Quéguiner S, Boscher E, Bougeard S, Ermel G, Salvat G, et al. Detection of *Listeria monocytogenes* in raw and pasteurized liquid whole eggs and characterization by PFGE. Int J Food Microbiol 2010;138:56–62.

Available online at www.sciencedirect.com