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*Original Article*

# Prevalence of *Listeria monocytogenes* in raw bovine milk and milk products from central highlands of Ethiopia

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## Abstract

Introduction: *Listeria monocytogenes* is of major significance in human and veterinary medicine. Most human *Listeria* infections are foodborne and the association of contaminated milk and dairy produce consumption with human listeriosis is noteworthy. In Ethiopia, there is limited data regarding the prevalence of *L. monocytogenes* in raw bovine milk and dairy products. The aim of this study was, therefore, to determine the prevalence of *L. monocytogenes* in raw bovine milk and dairy produce.

Methodology: A total of 443 milk and milk product samples were microbiologically analyzed following methods recommended by the U.S. Food and Drug Administration Bacteriological Analytical Manual to isolate *Listeria* spp.

Results: The overall prevalence of *Listeria spp*. was 28.4% and specifically that of *L. monocytogenes* was 5.6%. Taking the prevalence of *Listeria spp*. into consideration, cheese was found to be highly contaminated at 60%, followed by pasteurized milk samples (40%), raw milk (18.9%) and yoghurt (5%). Considering the prevalence of *Listeria monocytogenes* only, raw milk had the lowest contamination while cheese had the highest, followed by pasteurized milk and yoghurt.

Conclusions: Raw milk and milk products produced in urban and peri-urban areas of central Ethiopia were contaminated with pathogenic bacteria, *L. monocytogenes*. The detection of this pathogen in raw milk and milk products warrants an urgent regulatory mechanism to be put in place and also the potential role of milk processing plants in the contamination of dairy products should be investigated.

**Key words:** *Listeria monocytogenes;* milk products; bovine milk*;* Ethiopia.

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## Introduction

*Listeria monocytogenes* is of paramount significance in human and veterinary health [1]. Human acquisition of listeriosis from animal sources has been shown to occur as an occupational hazard especially in farmers, butchers, poultry workers and veterinary surgeons. On the other hand, most human infections are foodborne and the association of contaminated milk and dairy produce consumption with human listeriosis has been highlighted [2,3 4].

Starting in the 1960s, as a result of the introduction and widespread use of refrigerators, processed foods and extended shelf life foods became more associated with listeriosis due to *L. monocytogenes* [5]. This pathogen in addition to its zoonotic potential is also an important environmental contaminant of public health significance [6]. Various types of food were deemed to be potentially contaminated with this pathogen. The prevalence was found to differ from place to place, based on hygiene, food content and environmental contamination rate of the specific areas [6].

Reports indicated that *Listeria* spp. including *L. monocytogenes* is most frequently prevalent in the milk-processing environment including steps, drains and floors [7]. Even though pasteurization of raw milk destroys *L. monocytogenes*, this process does not eliminate later risk of contamination of dairy products [8]. In addition, a previous report from Finland indicated that dairy products that are actually made of pasteurized milk might become contaminated by *L. monocytogenes* during subsequent stages of production [9].

Of the total milk production in Ethiopia, it is reported that 71% to 97% of milk is consumed through

an informal market [10]. The milk in such cases is either consumed at home in raw form or traditionally processed into other dairy products. Furthermore, only less than one percent is consumed after pasteurizing [11]. In addition, there has not been a pre-set standard for milk quality in the country [10]. During the previous regime, the formal market only consisted of milk processed by government-owned Dairy Development Enterprise (DDE) nowadays known as *Lame* dairy enterprise [12]. However, to date there are close to a dozen privately owned milk processing enterprises in and around the capital of Ethiopia; for instance, there isa considerable number of established dairy co-operatives in Addis Ababa and its vicinity. The potential for expanding dairy co-operatives in these areas is also huge. The areas in which the co-operatives are established include: Addis Ababa, Holeta, Sululta, Selale, Sheno, Debre-Birhan, Adama (Nazareth), Arsi, and Debre-Zeit [13]. Among these, Debre-Zeit and Selale are well known for their established dairy co-operative unions and have been on top of others in terms of their success and growth in the dairy industry [14,15]. Asela Town has also a great potential for dairy development and is making progress in promoting a market-oriented system [16].

In Ethiopia, data regarding the prevalence of *L. monocytogenes* is limited. A study conducted in Addis Ababa city showed the prevalence of *Listeria spp*. to be 32.6% and that of *L. monocytogenes* to be 5.1% in some foods such as meat, cheese, fish, pork, poultry and ice cream [17]. Therefore, the aim of this study was to determine the prevalence of *L. monocytogenes* recovered from raw bovine milk and milk products (pasteurized milk, cheese and yoghurt) produced in urban and peri-urban areas of central highlands of Ethiopia.

## Materials and Methods

*Sampling areas and sample types*

Four small-scale bovine milk-producing co-operatives were selected conveniently from urban and per-urban regions (Asela, Debre-Zeit, Addis Ababa (Akaki/Kaleti) and Selale), which are the areas supplying milk to the capital city, Addis Ababa. In addition, different originally packaged milk products (n = 100) were also purchased from supermarkets representing different milk processing plants in and around Addis Ababa city. The study area included milk collection centers representing the milk-shed area of central Ethiopia. A total of 443 samples were analyzed which included: samples of raw on-farm pooled milk and bulk tank (n = 343), Pasteurized milk (n = 65), yogurt (n = 20) and cheese (n = 15).

*Culture and Identification*

Methods used to isolate *Listeria spp*. are as recommended by the U.S. Food and Drug Administration [18]. Raw/pasteurized milk samples (25ml) were enriched in 225mL of *Listeria* broth-LEB (Difco Laboratories, Detroit, USA) (1:9 V/V ratio) and after 4 hours of incubation, selective reagents (*Listeria* selective supplement, Difco) were added and the incubation step was carried out at 30°C for 48 hours. Aliquots were streaked onto Oxford agar plates (Difco) after 24 and 48 hours of incubation and kept at 35°C. Colonies with typical *Listeria* morphology were transferred to tryptic soy agar (TSA, Difco) with yeast extract and incubated at 30°C for 24 to 48 hours. After microscopical analysis of the isolates, confirmation was performed using biochemical results (catalase, xylose, mannitol and ramnose fermentation, and CAMP test using *Staphylococcus aureus* ATCC 25923). Twenty-five grams of milk products (cheese and yoghurt) were also analyzed in the same manner as the raw/pasteurized milk samples.

*Statistical analysis*

Data on the frequency of isolation of *Listeria monocytogenes* from milk and milk product samples was initially presented using descriptive statistics. The identification of associations between variables under investigation and the dependent variable were assessed by non-parametric tests for nominal variables using the Chi-square method. To analyze the data the SPSS version 20.0 was used.

*Ethical considerations*

This research proposal was approved by the Research and Ethical Review Committee of Aklilu Lemma Institute of Pathobiology (ALIPB) and ethically cleared by the Institutional Review Board-ALIPB, Addis Ababa University.

## Results

*Isolation of Listeria spp.*

Of the total samples processed (n=443), the overall prevalence of *Listeria spp*. was 28.4%. Categorizing the samples into raw milk and milk products, the overall other *Listeria spp.* prevalence was 18.9% (65/343) for raw milk and 36% (36/100) for milk products. The difference in this case was found to be significant ($p < 0.05$). In the case of *Listeria monocytogenes* alone, the prevalence was 2.04%

(7/343) for raw milk samples and 18% (18/100) for milk product samples. This difference was also found to be significant ($p < 0.05$).The proportion of the different *Listeria spp*. was in the order of: *Listeria innocua* (7.5%), *Listeria monocytogenes* (5.6%), *Listeria ivanovii* (4.3%), *Listeria grayi* (4.3%), *Listeria seeligeri* (2.7%), others (2.7%) and *Listeria welshimeri* (1.4%) (Table 1).

*Geographic distribution of Listeria species*

Different *Listeria spp*. prevalence was found among the various geographical areas under investigation. Considering the overall prevalence of *Listeria spp*., the two urban areas, Debre-Zeit and Addis Ababa, had the highest prevalence as compared to the peri-urban (Selale and Asela). This difference was statistically significant ($p < 0.05$). When taking the prevalence of *L. monocytogenes* only, the findings were also similar with samples from Addis Ababa having the highest prevalence (4.3%) followed by samples from Debre-Zeit (2.5%) and no Listeria strain was obtained from samples that were collected from Asela region. This difference, however, was not statistically significant.

When stratifying the regions into urban and peri-urban settings, the prevalence of *L. monocytogenes* was higher for the urban setting (3.4%) compared to peri-urban areas (1.03%) (Table 2), even though the difference was not statistically significant, and the same trend was also noted for the overall prevalence of the total *Listeria spp*., in this case, the difference was statistically significant ($p < 0.05$).

*Distribution of Listeria species among the different sample types*

In the current study, four different types of milk and milk products, which include raw milk (on farm pooled and combined bulk tank milk), pasteurized milk, yoghurt, and cheese were analyzed. The overall prevalence of *Listeria spp*. in the different types of samples is indicated in Table 1. Taking the prevalence of the whole *Listeria spp*. into consideration, cheese samples were found to be highly contaminated with 60% followed by pasteurized milk samples (40%), raw milk samples (18.9%) and the lowest prevalence was found in yoghurt samples (5%). When considering the prevalence of *L. monocytogenes* only, samples from cheese still showedthe highest prevalence of all

**Table 1.** Prevalence of *Listeria* spp. in the different sample types (raw milk, pasteurized milk, cheese and yoghurt), November 2011 - July 2012.

| ***Listeria* species** | **Sample types** | | | | |
|---|---|---|---|---|---|
| | **Raw milk No. (%) n=343** | **Pasteurized No. (%) n=65** | **Yoghurt No. (%) n=20** | **Cheese No. (%) n=15** | **Total No. (%) n=443** |
| *L. monocytogenes* | 7 (2.04) | 13 (20) | 1 (5) | 4 (26.7) | 25(5.6) |
| *L. innocua* | 22 (6.4) | 10 (15.4) | 0 (0) | 1 (6.7) | 33(7.4) |
| *L. ivanovii* | 12 (3.5) | 6 (9.2) | 1(5) | 0 (0) | 19(4.3) |
| *L. grayi* | 15 (4.4) | 2 (3.1) | 0(0) | 2 (13.3) | 19(4.3) |
| *L. seeligeri* | 8 (2.3) | 1(1.5) | 0(0) | 3 (20) | 12(2.7) |
| *L. welshimeri* | 2 (0.6) | 4 (6.2) | 0(0) | 0 (0) | 6(1.4) |
| [1]*Others* | 6 (1.7) | 3 (4.6) | 0(0) | 3 (20) | 12(2.7) |
| **Total** | **72 (20.99)** | **39 (60)** | **2 (10)** | **13 (86.7)** | **126(28.4)** |

[1] Others refer to isolates which could not be placed in any of the known species based on biochemical test.

**Table 2.** Distribution of *Listeria* species isolated in Urban and Peri-urban milk shed areas (*Selale, Asela, Debre-Zeit* and *Addis Ababa*), November 2011 - July 2012

| ***Listeria* species** | **Milk shed areas (no. of milk analyzed)** | | **Total** |
|---|---|---|---|
| | **Urban area[1] (n=149 ) No. (%)** | **Peri-urban area[2] (n=194 ) No. (%)** | **Both areas (n=343) No. (%)** |
| *L. monocytogenes* | 5(3.4 %) | 2(1.03%) | 7 (2.0%) |
| *L. innocua* | 16 (10.7 %) | 6(3.1%) | 22 (31%) |
| *L. ivanovii* | 6 (4.03 %) | 6 (3.1%) | 12 (3.4%) |
| *L. grayi* | 11 (7.4 %) | 4(2.1 %) | 15 (4.3%) |
| *L. seeligeri* | 6 (4.03%) | 2 (1.1%) | 8 (2.3%) |
| *L. welshimeri* | 2 (1.34%) | 0 (0%) | 2 (0.6%) |
| Others[3] | 5 (3.4%) | 1 (0.52%) | 6 (1.7%) |
| **Total** | **51(34.2%)** | **21 (10.8%)** | **72 (21%)** |

[1]Urban area =Addis Ababa and Debre-Zeit; [2]Peri-urban area =Asela and Selale; [3]Not differentiated at species level by biochemical tests

sample types followed by pasteurized milk samples, yoghurt samples and the lowest contamination was noticedin raw milk samples (Table 1). This difference in prevalence of both *L. monocytogenes* and the overall other *Listeria spp*. was statistically significant ($p < 0.05$).

## Discussion

Both raw bovine milk and milk product specimens in this study were contaminated with *Listeria* species. The overall prevalence for *Listeria spp*. (28.4%) reported in this study is slightly higher than prevalence observed by Mengesha *et al.* who isolated *Listeria spp* (26.6%) from ready to eat food that included: pasteurized milk, cheese, ice cream, and cakes in Addis Ababa, Ethiopia [19]. In the same study, they reported the prevalence of *L. monocytogenes* to be 4.8%, which is also lower than prevalence reported in the present study. However, findings of the present study are comparable with a previous study, which reported prevalence of *L. monocytogenes* in some foods, such as, meat, cheese, fish, pork, poultry and ice cream, to be 5.1% [17]. Gebretsadik *et al*. [20] also reported a 5.4% prevalence for *L. monocytogenes* from different food items analyzed, such as Ethiopian cottage cheese, raw beef, raw milk and liquid whole egg; with raw milk being the most contaminated one (13%). In Botswana, a total of 1,324 food samples obtained from supermarkets and street vendors were analyzed for the presence of *L. monocytogenes* of which 57(4.3%) were found positive. In this same study the *L. monocytogenes* contamination rate of different foods was in the order of 12.3%, 5.3%, 0%, 47.4%, and 35.1% from cheese, raw milk, meat, frozen cabbage, and salad, respectively [21]. In Algeria, some workers isolated *L. monocytogenes* from raw milk, whey and curdle milk and reported the prevalence of *L. monocytogenes* to be 2.61% from farm milk samples, 7.5% from tanker milk and 0% from curdle milk [22]. Such a relatively higher prevalence of *L. monocytogenes* in the current study poses high public health concern as most of the milk and milk products in Ethiopia are consumed in raw forms without being treated with sufficient heat. Considering the high fatality rate for listeriosis, especially in the risk groups, this rate of prevalence is of high public health concern [8].

Different rates of *Listeria spp*. prevalence were observed among the various geographical areas under investigation. Considering the overall prevalence of *Listeria spp*., Debre-Zeit had the highest rate followed by Addis Ababa and the lowest prevalence was observed for Selale. The significant difference observed between urban and peri-urban regions as to the prevalence of *Listeria spp*. may indicate that there could be different risk factors associated with contamination of milk between the two regions. A recent report from Brazil signified that there was a difference in contamination index of milk by a range of pathogens between two different geographical locations in which different risk factors such as temporary cattle confinement, low milk production, low milking machine cleaning frequency, and milk storage area without tile walls were identified specifically for each study region [23].

In the present study, it was observed that there was a difference in the rate at which *Listeria* species were recovered among the different sample types. The presence of these bacteria in processed milk products could be post-processing contamination. Contrary to our finding, a previous study from Ethiopia [19] did not isolate any of the *Listeria spp*. in pasteurized milk even though such possibilities have been reported elsewhere [24]. The possible explanation for this discrepancy could be due to difference in isolation methods and time period of the study conducted and also difference in sample sources. In this case, the prevalence of the overall *Listeria spp*. varied significantly between raw milk samples and milk product samples. For instance, in the case of *L. monocytogenes* the contamination rate was 2.04% for raw milk and 18% for the milk products (pasteurized milk, cheese and yoghurt). This does not mean that raw milk is safer than milk products; however the finding emphasizes the potential role of milk products (specifically, pasteurized milk and cheese) in the public health. In fact, pasteurization does not in any way guarantee the absolute safety of milk and milk products. This is exemplified by a report from the USA, State of Massachusetts, where drinking pasteurized milk was associated with an outbreak of *L. monocytogenes* infection in 2007 [25]. Though it may be rare, the potential for pasteurized milk in causing a listeriosis outbreak always has to be considered, especially in countries like Ethiopia where there is a lack of a strong regulatory system.

When considering the prevalence of *L. monocytogenes* alone, samples from cheese still had the highest contamination rate of all sample types, followed by pasteurized milk samples, yoghurt samples and the lowest contamination was observed for raw milk samples. The high level of contamination of cheese samples in the current study may be associated with either the actual production processing

of the cheeses, or due to the post-processing contamination of the product, probably while it is ripening in the ripening rooms. As cheese is usually left refrigerated for longer time, contaminated cheese in this case could have a significant health implication. In addition, the majority of cases of sporadic human listeriosis foods associated with transmission are found to be predominantly ready to eat, with extended shelf life that gives a chance for supporting *L. monocytogenes* growth [26].

The presence of *L. monocytogenes* in cheese and pasteurized milk is a public health hazard for consumers due to the fact that dairy products have been implicated in many human listeriosis outbreaks both as invasive and non-invasive forms [27-29]. There is a universal trust among the general public that pasteurized milk and milk products from processing plants are safe and therefore, most individuals in urban areas consume pasteurized milk and dairy products (cheese; yoghurt) with seemingly no knowledge of the possibility of post-production contamination of these products. Field observation in sale points of some of the milk processing plants in Addis Ababa indicated that a considerable number of consumers were seen to drink pasteurized milk directly from these shops with a full trust that the processing plant provides safe milk. As it is obvious from the result of the present study, it is necessary to have a regulatory mechanism over the dairy processing plants regarding the safety of their products. The presence of *L. monocytogenes* at such rate in processed dairy products sends a critical message to the public health officials and concerned food safety regulatory bodies in Ethiopia. Therefore, the role of milk processing plants in the contamination of dairy products by *L. monocytogenes* in Ethiopia, which was not addressed in the current study, needs to be further investigated.

## Conclusion

The result from this study revealed that milk and milk product contamination with *L. monocytogenes* in the central highlands of Ethiopia is an issue of public health significance. The presence of *L. monocytogenes* in raw bovine milk and especially milk products such as pasteurized milk warrants an urgent regulatory mechanism to be put in place.

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