

ASSESSMENT OF *LISTERIA MONOCYTOGENES* PRESENCE IN RAW MILK SAMPLES AND CERTAIN CHEESE VARIETIES

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ABSTRACT

Listeria monocytogenes has been considered a significant food-borne pathogen in recent years, representing a major concern for public health and food safety. Consequently, the objective of the current investigation was to assess the prevalence of *L. monocytogenes* and other *Listeria* species in raw milk and certain cheese varieties. The presence of *L. monocytogenes* was screened in a total of 769 dairy samples, which were randomly collected from farms and dairy shops located in Cairo and Giza governorates. The samples included 212 raw milk samples from farms, 307 raw milk samples from dairy stores, and 250 cheese samples, including Kareish, Talaga, Feta, Processed, Ras, Gouda, and Cheddar (25 each). In total, twenty-two of the analyzed samples were contaminated with *Listeria* species, representing 2.86%. Additionally, there were only five positive samples for *L. monocytogenes*, accounting for 0.65%. The prevalence of *Listeria* spp. in the examined milk samples from dairy shops, farms, and cheese was 6.17%, 0.47%, and 0.8%, respectively. Depending on the outcomes of the biochemical identification assays, *L. monocytogenes* was detected in 1.30% and 0.47% of the examined milk samples from dairy shops and farms, respectively, while it couldn't be isolated from all cheese samples. *L. monocytogenes* isolates were confirmed positive using Polymerase Chain Reaction (PCR) targeting the hemolysin (*hly A*) gene. Furthermore, *L. ivanovii* and *L. grayi* were isolated from two samples of Kareish cheese representing 8%. In conclusion, the obtained results indicated a potential risk of milk and cheese contamination with *Listeria*. Thus, strict hygienic measures and frequent investigations must be applied to control such microorganisms.

Keywords: *Listeria*, Milk, Cheese, *L. monocytogenes*, *L. ivanovii*, *L. grayi*

INTRODUCTION

Foodborne listeriosis is a significant global health concern that is closely associated with the rise in global trade and travel. Foodborne listeriosis is a rare illness that is linked to the consumption of

contaminated food (Ryser 2021, Saleh *et al.*, 2024), and was first identified as a foodborne illness in 1980 (Schlech *et al.*, 1993, Schlech *et al.*, 1994).

Listeria species are widely distributed globally and have been isolated from a variety of sources, including water, food, such as milk, meat, and vegetables, as well as environmental sources, such as contaminated silage, feces, and sewage (Molla *et al.*, 2004, Rahimi *et al.*, 2010, Osman *et al.*, 2019). *Listeria*

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monocytogenes is a facultative intracellular, gram-positive foodborne pathogen characterized by its psychotropic and ubiquitous nature, as well as its ability to survive and proliferate in a wide range of harsh environments and foods. Pregnant women and immunocompromised individuals are at higher risk for listeriosis. In people with no predisposing factors, invasive listeriosis is rare, and the most typical symptom is mild gastroenteritis with fever, headache, nausea, diarrhea, and abdominal pain (Silk *et al.*, 2013). Several outbreaks of listeriosis have been linked to the consumption of contaminated milk. These outbreaks can affect a large number of individuals, with an overall mortality rate of around 30% (Abdeen *et al.*, 2021). In addition, *L. monocytogenes* has been detected in a wide range of dairy products, including cheese, which is the most extensively examined product due to its known association with foodborne listeriosis. The presence of *L. monocytogenes* in food remains possible at any stage of food production, packaging, storage, and distribution (Michard and Jardy 1989, Renterghem *et al.*, 1990).

As a result, the ability of *L. monocytogenes* to persist and proliferate in food-processing environments at low temperatures poses a significant threat to public health (Saleh *et al.*, 2024). Given the potential risk of *L. monocytogenes* contamination in raw milk and cheese, this study aims to evaluate the occurrence of *Listeria* species, particularly *L. monocytogenes*, in raw milk and cheese varieties commonly consumed in Egypt. The findings of this study will enhance our understanding of the prevalence of this foodborne pathogen in these dairy products and inform food safety measures to better protect public health.

MATERIALS AND METHODS

Samples collection:

Between August 2022 and November 2023, a total of 519 raw milk samples (212 from

dairy farms and 307 from dairy shops) and 250 samples of cheese varieties including Kareish, Talaga, Feta, cheese, Ras, Gouda, and Cheddar (25 each) were collected from dairy shops and street vendors in different localities in Cairo and Giza governorate. All samples were randomly collected and transported under aseptic conditions in an insulated ice box and evaluated upon their arrival.

Isolation of *Listeria* species from the examined samples.

A standard method of *Listeria* isolation was implemented according to the protocol described by (ISO 1996). Briefly, 25ml/g of each milk and cheese sample was pre-enriched on 225 ml of half Fraser broth (Himedia) and incubated at 30°C/24 hrs. Subsequently, 0.1 ml from the pre-enrichment culture was transferred into 10 ml of Fraser broth (Himedia) and incubated at 37°C/ 48 hrs. Following the enrichment stage, a loopful of cultured Fraser broth was streaked onto Oxford agar plates (Oxoid) and incubated at 37°C/24-48 hrs. Plates were then examined for typical *Listeria* colonies. Suspicious colonies were transferred onto tryptic soy agar with 0.6% yeast extract (TSA-YE, Himedia) and incubated at 37°C/24 hrs. Subsequently, they were maintained at 4 °C for further identification. All the isolated strains (n= 27) were identified through the application of biochemical assays, such as, Gram staining, catalase test, motility test at 25 and 37 °C, nitrate reduction, MR/VP test, β-haemolysis activity, CAMP test, and acid production from glucose, mannitol, rhamnose, and xylose.

Polymerase chain reaction (PCR).

DNA extraction (Bansal 1996):

Each isolate of the previously identified *L. monocytogenes* was streaked onto tryptic soy agar with 0.6% yeast extract (TSA-YE, Himedia) and incubated for 24 hrs at 37°C. Then colonies were collected into Eppendorf tubes containing 1 ml phosphate buffered saline (PBS) (Himedia) and DNA extraction of *L. monocytogenes* was done

by the boiling method. Briefly, bacterial colonies were washed once with 1 ml PBS/pH 7.4, re-suspended in water, and kept in a boiling water bath for 10 min. The PCR reaction was conducted using the clear supernatants that were obtained after a five-minute centrifugation at 12000 g.

PCR reaction:

DNA samples were amplified in 25µl of the following reaction mixtures: 2 µl of DNA

template, 1µl of 20 pmol of each primer, and 5 µl of 5X of PCR master mix in separate reactions. The reaction conditions were optimized to be 95°C for 5 min as initial denaturation followed by 30 cycles of 95 °C/15 sec, 57 °C /2 sec, 72 °C /30 sec, and final extension cycle at 72 °C /5 min. Primer set used (*hly A*) : LM1 CCT-AAG-ACG-CCA-AT C-GAA and LM2 AAG-CGC-TTG-CAA-CTG-CTC (702bp) (Bansal 1996).

Tables and Figures:

Table 1: The incidence of *Listeria* species in milk samples

<i>Listeria</i> spp.	Raw milk from dairy shops (N=307)		Raw milk from farms (N=212)		Total (N=519)	
	Positive samples					
	No	%	No	%	No	%
<i>L.monocytogenes</i>	4.0	1.30	1.0	0.47	5.0	0.96
<i>L.ivanovii</i>	1.0	0.32	ND	-	1.0	0.19
<i>L.seeligeri</i>	1.0	0.32	ND	-	1.0	0.19
<i>L.innocua</i>	2.0	0.65	ND	-	2.0	0.38
<i>L.welshimeri</i>	4.0	1.30	ND	-	4.0	0.77
<i>L.grayi</i>	2.0	0.65	ND	-	2.0	0.38
<i>L.murrayi</i>	5.0	1.63	ND	-	5.0	0.96
Total	19.0	6.17	1.0	0.47	20.0	3.83

ND: not detected

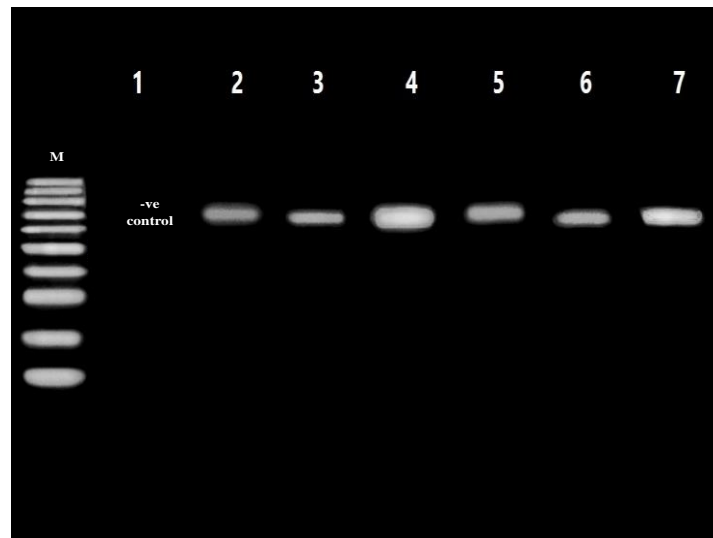
Table 2: The occurrence of *Listeria* species in the examined cheese samples

	Kareish (N=25)		Talaga (N=25)		Feta (N=25)		Processed (N=25)		Ras (N=25)		Gouda (N=25)		Cheddar (N=25)		Total (N=250)	
	Positive samples															
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
<i>L.ivanovii</i>	1.0	4.0	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-	1.0	0.4
<i>L.grayi</i>	1.0	4.0	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-	1.0	0.4
Total	2.0	8.0	-	-	-	-	-	-	-	-	-	-	-	-	2.0	0.8

ND: not detected

Table 3: The prevalence of *Listeria* species and *L. monocytogenes* in the examined food samples

Type of samples	No. of samples	<i>Listeria</i> spp.		<i>L. monocytogenes</i>	
		Positive samples			
		No	%	No	%
Raw Milk from dairy shops	307	19	2.47	4	0.52
Raw Milk from farms	212	1	0.13	1	0.13
Cheese	250	2	0.26	ND	-
Total	769	22	2.86	5	0.65

Figure 1: PCR for *L. monocytogenes* identification

L. monocytogenes specific band is 702bp,

Lane M: 100 bp DNA ladder,

Lane 1: Negative control,

Lane 2: positive *L. monocytogenes* control,

Lanes 3-7: positive *L. monocytogenes* from raw milk samples.

RESULTS

The data in Table 1 represented the presence of various *Listeria* species in the analyzed milk samples. *L. monocytogenes* was identified in 4 milk samples obtained from dairy shops, representing 1.30% of the samples analyzed. Additionally, it was detected in a single milk sample obtained from farms, representing merely a 0.47% of the total samples. There was a total of 5 incidences of *L. monocytogenes* found, which accounted for 0.96% of all milk samples that were investigated. Additional *Listeria* species, including *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, *L. grayi*, and *L. murrayi*, were also detected in the milk samples obtained from dairy stores. However, their occurrence was less frequent, ranging from 0.32% to 1.63%. Only two strains of the *Listeria* species were found in two samples of Kareish cheese out of the 250 samples that were tested. The tested cheese samples contained *L. ivanovii* and *L. grayi*, which accounted for 0.8% of the samples. However, *L. monocytogenes* was absent in all of the examined cheese samples. Table 3 provides a summary of the distribution of *Listeria* species in food

samples. It also presents the percentage of these species in the examined samples. Figure 1 shows the PCR result of confirmed *L. monocytogenes* isolates from milk samples being investigated, five positive bands (702bp) of *hly A* gene were confirmed (4 milk samples from dairy stores and one from dairy farm).

DISCUSSION

Listeriosis is an emerging zoonotic disease of significant global concern. It is a severe and often fatal infection primarily caused by the bacterium *L. monocytogenes* (Chlebicz and Śliżewska 2018). Certain food items, particularly dairy products, are more susceptible to *Listeria* contamination due to their nutritional composition, handling characteristics, and unfavorable storage conditions, making them a major source of foodborne listeriosis (Schlech 2000, Koopmans *et al.*, 2023). Foodborne listeriosis can lead to a serious and potentially life-threatening infection (Lepe 2020, Ryser 2021).

The environmental conditions of farms are conducive to the proliferation of *Listeria*.

This bacterium can thrive in a variety of environments, including soil, water, marshy and dusty conditions, and dams (Lungu *et al.*, 2009). Additionally, *Listeria* infections can cause mastitis in cows, although the incidence rate of subclinical mastitis appears to be lower for *L. monocytogenes* than for other mastitis-causing pathogens (Rawool *et al.*, 2007, Rodriguez *et al.*, 2021). Consumption of improperly fermented silage is another concern, as *Listeria* can proliferate in such substandard feed (Nucera *et al.*, 2016).

The results presented in Table (1) showed that 20 samples of the examined raw milk were positive for *Listeria* species (3.83%). *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, *L. grayi* and *L. murrayi*, were isolated in varying percentages while, *L. monocytogenes* was detected in one farm milk (0.47%) and four milk samples from dairy stores (1.30%). A higher prevalence (6%) was recorded in Menoufiya governorate, Egypt in the study applied by (Abdeen *et al.*, 2021) and (El-Demerdash and Raslan 2019) who found *L. monocytogenes* in 10% of the examined raw milk in urban and rural areas of Egypt. The level of *L. monocytogenes* in milk samples from dairy shops was virtually identical to that detected by (Tantawy, 2011) in Alexandria governorate, Egypt, where it was found in 3% of the tested samples.

The occurrence of *L. monocytogenes* in the examined milk samples from dairy shops (1.30%) was higher than that in the examined milk samples from farms (0.47%). The results confirmed that the primary issues with the epidemiology of listeriosis are of environmental origin, rather than being related to the animal itself, as previously mentioned (Lucchini *et al.* 2023). The presence of *Listeria* spp. in the examined raw milk samples may be attributed to contamination of milk from the producing animal, spoiled silage, feces, inadequate cleaning of cow's exercise area, poor cow cleanliness, inadequate cleaning of milking utensils and equipment, as well

as due to poor hygienic measures applied during milking, handling, transportation and distribution (Jamali *et al.*, 2013, Lee *et al.*, 2019, Oluwafemi *et al.*, 2023). Using PCR targeting *hly A* gene, five samples were confirmed as *L. monocytogenes*. Four samples (1.30%) from the milk samples that collected from dairy shops and one sample (0.47%) from the farms (Figure 1).

Consuming contaminated food products, such as processed meat, dairy products (raw milk, cheese, butter), ready-to-eat food, fresh vegetables, and fruits, is nearly the sole cause of human listeriosis (Vidovic *et al.*, 2024). After the identification of *L. monocytogenes* as a foodborne pathogen in the 1980s, comprehensive food safety measures were put in place both nationally and internationally (Schlech *et al.*, 1983). *L. monocytogenes* can survive and proliferate in harsh conditions with a pH ranging from 4.6 to 9.5 and salt concentrations of up to 10.0% (Carpentier and Cerf 2011, Ferreira *et al.*, 2014). The physico-chemical characteristics of fresh cheese are often conducive to the proliferation of bacteria. These characteristics include a high moisture content (>50%), an average pH level above 6, and a comparatively low salt content (0.85%) (Kapetanakou *et al.*, 2017).

The findings in Table (2) showed that the occurrence of *Listeria* spp. in different types of cheese purchased from dairy shops and street vendors in Cairo and Giza was 0.8 % in the examined Kareish cheese while the other tested samples of Talaga, Feta, processed, Ras, Gouda and Cheddar cheese were listeria-free. *L. ivanovii* and *L. grayi* were isolated from Kareish cheese. While *L. monocytogenes* couldn't be isolated from all analyzed cheese types.

Concerning Kareish cheese, a nearly similar incidence (1%) was observed by (Elafify *et al.*, 2022). Our results were lower than those detected by (Dapgh *et al.* 2020) who detected the existence of *Listeria* spp. in some Egyptian food and recorded 14 positive out of 70 (20%) Kareish cheese

samples from Giza, Egypt and (Meshref *et al.*, 2015) who found 13.73% *Listeria* in Kareish cheese in Beni-Suef governorates, Egypt. They contributed the relatively high incidence of *Listeria* spp. contamination in Kareish cheese to the use of unpasteurized milk, high water activity, environmental contamination, and inadequate production and poor personal hygiene.

In contrast, hard and semi-hard cheeses generally have a lower incidence of *Listeria* contamination, which is likely due to their decreased water activity (aw) creating unfavorable conditions for *Listeria* growth and survival (Melo *et al.*, 2015, Gérard *et al.*, 2018, Martinez-Rios and Dalgaard 2018). In Egypt, (Saleh *et al.*, 2021) isolated *Listeria* spp. (*L. innocua* then *L. ivanovii*, and *L. monocytogenes*) from six Ras cheese samples out of 50 (12%) while (Mohamed *et al.* 2020) couldn't isolate the microorganism from Ras cheese.

CONCLUSION

The results of the present study demonstrated the spatial distribution and presence of *L. monocytogenes* and other *Listeria* spp. in fresh milk and certain cheese varieties in the governorates of Cairo and Giza, Egypt. These results indicate that raw (non-heat treated) milk may act as a reservoir for *L. monocytogenes*, thereby representing a potential hazard to consumers. In the context of immunodeficient people, the presence of this microorganism in food products could potentially be a threat to public health. This investigation emphasized the importance of implementing hygienic practices throughout the entire food chain, from production to consumption, to improve food safety. Furthermore, consumers must know the health hazards associated with the consumption of raw foods and the appropriate thermal treatment procedures for food to prevent the transmission of such severe infections.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

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تقييم وجود الليستيريا مونوسيتوجينز في عينات الحليب الخام وبعض أصناف الجبن

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تعتبر الليستيريا المستوحدة *Listeria monocytogenes* من مسببات الأمراض الخطيرة التي تنقلها الأغذية في السنوات الأخيرة ، مما يمثل مصدر قلق كبير للصحة العامة وسلامة الأغذية. وبالتالي ، كان الهدف من إجراء هذه الدراسة هو تقييم انتشار الليستيريا مونوسيتوجينز وأنواع الليستيريا الأخرى في الحليب الخام وبعض أنواع الجبن. تم فحص وجود الليستيريا مونوسيتوجينز في ٧٦٩ عينة ألبان ، والتي تم جمعها عشوائياً من المزارع ومحلات الألبان الموجودة في القاهرة والجيزة. وشملت العينات ٢١٢ عينة حليب خام من المزارع، و٣٠٧ عينات حليب خام من محلات الألبان، و٢٥٠ عينة جبن بما في ذلك القريش والثلاجة والفيثا والجبن المطبوخ والراس والجودة والشيدر (٢٥ لكل منهم). إجمالاً ، كانت اثنتان وعشرون عينة من العينات التي تم تحليلها ملوثة بأنواع الليستيريا المختلفة ، وهو ما يمثل ٢,٨٦٪. بالإضافة إلى ذلك ، لم يكن هناك سوى خمس عينات إيجابية لميكروب الليستيريا مونوسيتوجينز ، وهو ما يمثل ٠,٦٥٪. كان انتشار الليستيريا في عينات الحليب المفحوصة من محلات الألبان والمزارع والجبن ٦,١٧٪ ، ٠,٤٧٪ ، ٠,٨٪ ، على التوالي. اعتماداً على نتائج التحليلات البيوكيميائية ؛ تم الكشف عن الليستيريا مونوسيتوجينز في ١,٣٠٪ و ٠,٤٧٪ من عينات الحليب التي تم فحصها من محلات الألبان والمزارع ، على التوالي بينما لم يتم عزلها من جميع عينات الجبن التي تم فحصها. تم تأكيد أن عزلات الليستيريا مونوسيتوجينز إيجابية باستخدام تفاعل البوليميراز المتسلسل (PCR) الذي يستهدف جين الهيموليزين (hly A). علاوة على ذلك ، تم عزل الليستيريا ايفانوفي والليستيريا جراي من عينتين من جبن القريش مما يمثل ٨٪. في الختام ، أشارت النتائج التي تم الحصول عليها إلى أن قد يكون هناك خطر محتمل لتلوث الحليب وبعض أنواع الجبن المنتج في الأسواق بميكروبات الليستيريا ولهذا فلا بد من وضع قواعد صحية صارمة و الفحص الدوري للسيطرة على هذه الميكروبات.