ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 4, pp: (19-25), Month: October - December 2017, Available at: www.researchpublish.com

Prevalence of *Listeria* spp. among Dairy, Meat and their Products Marketed in Tripoli, Libya

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Abstract: **The prevalence of *Listeria* species in retail raw animal food products covering most Tripoli city in Libya were studied with testing of 180 samples of dairy, meat and their products from September 2015 to May 2016. Among all investigated samples 79 (43.8%) tested positive for *Listeria* spp, 32 (40%) samples were positive from different dairy products (7 laben, 9 raw cow's milk, 8 Ricotta cheese, 8 Maassora cheese) and 47 (47%) samples from various meat and its products (9 chicken meat, 12 chicken burger, 3 raw beef, 12 beef burger, 11 beef sausage). A total of 158 isolates were analyzed and confirmed by biochemical characterization and hemolysis on sheep blood agar which revealed that 22 (13.9%) isolates were *Listeria welshimeri*, 54 (34.2%) were *L. murrayi*, 12 (7.6%) were identified *as L. grayi,* 23 (14.6%) were *L. momcytogenes* and 47 (29.7%) were *L. innocua.* Neither *L. ivanovii* nor *L. seeligeri* were detected. *Listeria spp.* were isolated as follows: 17 (10.8%) from raw milk, 10 (6.3%) Laben, 12 (7.6%) Massora cheese, 25 (15.8%) chicken meat, 41 (25.9%) raw beef, 11 (7%) beef burger. While 14 (8.7%) was isolated from Ricotta cheese, chicken burger and beef sausage. The data obtained in this study provides useful information for assessment of the possible risk posed to Libyan consumers and will have a significant public health impact in Libya. This is the first study reporting the occurrence and distribution of *Listeria* spp. in retail dairy, meat and their products purchased in Tripoli city, Libya.**

Keywords: ***Listeria*** **spp., Milk, Dairy Products, Meat, Meat products, Libya.**

1. INTRODUCTION

Listeriosis is one of the important emerging bacterial zoonotic diseases that occurs in a variety of animals used for food (particularly in cattle, sheep and goats) and humans. It causes meningitis, encephalitis, miscarriage and septicemia [1],[2]. It arises mainly from the consumption of contaminated animal food products [3],[4]. Various studies have shown that people at greater risk are pregnant women and fetus, alcoholics, drug abusers, patients with corticosteroid therapy, AIDS patients and the elderly [5],[1]. Infection acquired in early pregnancy may lead to abortion, stillbirth or premature delivery. When listeriosis is acquired late in pregnancy it can be transmitted transplacentally and lead to neonatal listeriosis [4],[1].

Several reports have implicated food types such as milk and milk products, meat and meat products, raw vegetables and sea foods as sources of foodborne listeriosis [6],[7],[8]. According to [5] listeriosis has emerged to be more important in developed countries than in developing countries. This could be due to 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 Libya. There is comparatively little information concerning foodborne listeriosis as well as insufficient clinical data are available in country such Libya. Unlike most pathogenic, foodborne bacteria, *Listeria* spp. have unique physiological characteristics that allow growth at refrigeration temperature. The organism can also tolerate a pH between 5.4 and 9.6 [9]. Among the different species of the genus *Listeria*, *L. monocytogenes* has been known to cause listeriosis in humans and animals [10],[11]. *L. monocytogenes* and other

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Listeria spp. have been reported from different types of food and clinical samples worldwide [9],[12],[13],[14],[2],[15]. Most cases of listeriosis tend to be foodborne, and a number of food items can become contaminated by *L. monocytogenes*, including raw milk, raw chicken meat, raw minced meat and soft cheese. Therefore, the aims of this study were to determine the occurrence of *Listeria* spp. in raw milk, meat, poultry meat and its products and to estimate the presence of *L. monocytogenes* and other *Listeria* strains in tested food products.

2. MATERIALS AND METHODS

2.1. Food Samples:

A hundred and eighty samples of animal origin consisting of twenty each of: raw milk, Maassora cheese, Ricotta cheese, Laben (Locally produce butter milk), raw chicken meat, chicken burger, raw beef, beef burger, beef sausage samples were purchased randomly from municipally licensed retail shops, butchers, parlors and markets of different localities of Tripoli city in Libya. The samples were kept in an icebox containing ice packs and immediately transported to the Food Hygiene laboratory of Faculty of Veterinary Medicine, University of Tripoli.

2.2. Samples Preparation and Inoculation:

The ISO 11290 method was employed to isolate the *Listeria* spp. [16], whereby pre-enrichment of 25 g sample was added to 225 mL half strength Fraser broth containing selective supplements (Liofilchem, Italy) and homogenized using a laboratory blender (Stomacher 400, Seward, England) at 240 rpm for 2 min then incubated for 24 h at 30 °C, which was followed by second enrichment of 0.1 mL of pre-enriched Fraser broth content in 10 mL full strength Fraser broth containing selective supplements (Liofilchem) for 48 h at 37 °C. After the enrichment procedure, a loopful of enriched full strength Fraser broth was streaked onto different selective agar, Listeria PALCAM agar (Liofilchem) and Listeria Oxford agar (Oxford formulation) (Liofilchem) and incubated for 48 h at 37 °C.

2.3. Isolation and Identification:

Three to five presumptive colonies from PALCAM agar (gray-green colonies surrounded by diffuse black zone) and Oxford agar plates (grayish, enriched with black zone-brown colonies with esculin hydrolysis halo) were re-streaked onto Tryptic Soya agar (Oxoid, Hampshire, UK) supplement with 0.6% yeast extract (Oxoid) (TSYEA) for further identification. Subsequently, pinpoint colonies on TSYEA were subjected to identification procedures [17] which included Gram's staining followed by a microscopic examination, catalase, oxidase tests and Henry's technique 45° transillumination for typical *Listeria* colonies and motility test (umbrella appearance) as discussed by [18]. For further confirmation of the isolates a 10% aqueous stock solution of Mannitol, L-Rhamnose and D-xylose were inoculated as described by [19]. CAMP test was carried out on the basis of the method described by [20] (Table 1).

TABLE 1: Biochemical test pattern and *in-vitro* pathogenicity profile of the *Listeria* species

Species identified	Biochemical tests					Sugar fermentation pattern			Hemolysis on 5% SBA	CAMP test with *S. aureus* (S) and *R. equi* (R)
	C	O	MR	VP	Ni	L-RH	D-Xy	D-Ma		
L. monocytogenes	+	-	+	+	-	+	-	-	+	+(S)
L. seeligeri	+	-	+	+	-	-	-	-	+	+(S)
L. welshimeri	+	-	+	+	-	+	+	-	-	-
L. innocua	+	-	+	+	-	+/-	-	-	-	-
L. grayi	+	-	+	+	-	-	-	+	-	-
L. murrayi	+	-	+	+	-	+/-	+/-	+	-	-
L. ivanovi	+	-	+	+	-	-	-	-	+	+

C=Catalase, O=Oxidase, Ni=Nitrate, L-Rh=Rhamnose, D-Xy=Xylose, D-Ma= Mannitol, SBA=Sheep blood agar, CAMP=Christie Atkins Munch Peterson, MR=Methyl red, VP=Voges Proskauer

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3. RESULTS

Out of 180 animal food product samples analyzed, 79 (43.8%) were positive for Listeria. *Listeria* spp. were isolated from raw cow's milk, Laben, Ricotta cheese, Maassora cheese, chicken meat, chicken burger, raw beef, beef burger and beef sausage samples (Table 2). The level of contamination of food samples by *Listeria* spp. varied and was high in beef and chicken burgers (60%), followed by beef sausage (55%), chicken meat and Ricotta cheese (45%), raw cow's milk and Maassora cheese (40%), laben (35%), while in raw beef was (15%).

The obtained results are summarized in table 2 and 3. A total of 158 presumptive *Listeria* spp. were isolated from 180 samples (raw cow milk, traditional dairy products, meat and its product). The total prevalence of positive samples was 43.8%. These isolates were identified as: *L. monocytogenes*, 23 (14.6%), *L. innocua*, 47 (29.7%), *L. welshimeri*, 22 (13.9%), *L. murrayi*, 54 (34.2%) and *L. grayi*, 12 (7.6%). *L. ivanovii* and *L. seeligeri* were not detected in this study (Table 3). The majority of *Listeria* spp. 66.4% (105/158) was isolated from meat samples and 33.5% (53/158) were found in dairy samples. The prevalence of *Listeria* spp. was found to be 47% (47/100) in meat samples (chicken meat and burger, raw beef, beef burger and sausage) in which *L. murrayi* was the most prevalent species recovered 38 (36.2%), followed by *L. innocua* with 30 (28.6%), *L. monocytogenes* with 16 (15.2%), *L. welshimeri* with 13 (12.4%) and *L. grayi* with 8 (7.6%) (Table 3). The prevalence of *Listeria* spp. in dairy samples was 40% (32/80). Raw milk was the most contaminated dairy product tested 7 (13.2 %) for *L. innocua*, 5 (9.4%) for *L. murrayi*, 4 (7.5 %) for *L. monocytogenes* and 1 (1.9 %) for *L. welshimeri*). While, Laben was less contaminated product with 3 (5.7 %) for both *L. innocua* and *L. welshimeri*, 2 (3.8%) for *L. murrayi* and 1 (1.9%) for both *L. grayi* and *L. monocytogenes* (Table 3). The prevalence of *Listeria* spp. in both cheeses (Maassora and Ricotta) was found to be similar (Table 2).

TABLE 2: Prevalence of *Listeria* spp. in different food of animal origin

Samples	**Number of Samples**		
	Examined	**Positive**	**%**
Raw cow milk	20	9	45
Laben	20	7	35
Ricotta cheese	20	8	40
Maassora cheese	20	8	40
Total	80	32	40
Chicken meat	20	9	45
Chicken burger	20	12	60
Raw beef	20	3	15
Beef burger	20	12	60
Beef sausage	20	11	55
Total	100	47	47
Total	**180**	**79**	**43.8**

TABLE 3: Prevalence of isolated *listeria* species in different dairy and meat product samples

Product		***Listeria* species**					
		L. grayi	***L. innocua***	***L. monocytogenes***	***L. murrayi***	***L. welshimeri***	**Total (%)**
Dairy	Raw milk	0	7	4	5	1	17 (10.8)
	Laben	1	3	1	2	3	10 (6.3)
	Maassora cheese	2	3	1	4	2	12 (7.6)
	Ricotta cheese	1	4	1	5	3	14 (8.7)
Meat	Chicken meat	2	8	4	7	4	25 (15.8)
	Chicken burger	0	4	3	5	2	14 (8.7)
	Raw beef	3	13	4	17	4	41 (25.9)
	Beef burger	2	3	1	4	1	11 (7)
	Beef sausage	1	2	4	5	2	14 (8.7)
Total (%)		**12 (7.6)**	**47 (29.7)**	**23 (14.6)**	**54 (34.2)**	**22 (13.9)**	**158**

4. DISCUSSION

Listeriosis has been recognized to be one of the emerging zoonotic diseases during the last three decades and is contracted mainly from the consumption of contaminated foods and food products [14],[1],[21]. Listeriosis is also considered as a serious disease for public health. Hence food industry and food laboratories should direct a special attention to such disease. The eating habits of Libyan people are different from those in western countries. The majority of people prefer to consume traditionally produced foods. Traditional dairy products in Libya are produced in small productive centers mostly located in urban areas and distributed unpacked. These products may be produced from unpasteurized milk. There have not been comprehensive studies performed on food contamination by *Listeria* and listeriosis in most developing countries. Providing such a data can convince regulatory authorities to set better codes of practice in the food industry and food distribution chain. In this case, Libya is no exception; however, these data are not enough to draw a conclusion on the risk assessment of *Listeria* in Libyan food customer. Furthermore, most cattle and sheep farms in Libya do not have adequate hygiene precautions and animals live in a natural environment together with people. Therefore, the study aimed to determine the prevalence of *Listeria* spp. in food of animal origin in Tripoli-Libya.

In the current study, 43.8% of all samples were positive for *Listeria* spp. of which *L. monocytogenes, L. innocua, L. murrayi, L. welshimeri* and *L. grayi* were isolated from 14.6%, 29.7.%, 34.2%, 13.9% and 7.6% of the tested samples, respectively.

Meat and its product have shown a higher level of contamination with *Listeria* spp. (47%), this is with agreement with study conducted by [22]. In addition, [23] reported that meat products showed the highest level of contamination while cheese was least contaminated and chicken meat was intermediate between these. These results indicated that meat products are considerably more likely to be contaminated with *Listeria* than other food products. This could be due to preparing and processing of minced meat (used, for example, in burger) in poor hygienic conditions [24]. Although other factors such as equipment and food additives can also have a role in contamination, burgers tend to be well cooked prior to consumption, reducing the chance of acquiring listeriosis in this kind of food [24].

In the present investigation, 40% of the traditional dairy product samples examined were positive for *Listeria* spp. Among all dairy products tested in this study, raw milk had the highest incidence of *Listeria* spp. There have been several studies suggested that raw milk is one of the most common paths for transmission of this organism, mainly due to sick animals on the farm and healthy animals are often carriers of *L. monocytogenes* and as such can be source of contamination of the environment, or milk. This could be referred to poor quality of prepared silage and fecal contamination during the transportation of milk and its storage [25], [26]. The prevalence of *L. monocytogenes* in raw milk samples found in this study is comparable with the results in other different countries on raw milk. For instance, [27],[28] have reported that about 6% prevalence of *L. monocytogenes* in bovine raw milk produced in Morocco and Algeria respectively. Similar results found by [29], where the incidence of contamination was 5% in milk and dairy products sold in mainland Portugal [30] reported also a low incidence of 3.6% of *L. monocytogenes* in raw milk produced in Spain. Whereas in China, the prevalence of *L. monocytogenes* in raw milk is about 1.2% [31]. The prevalence of *L. monocytogenes* contamination in Laben, Maassora and Ricotta cheeses was low (10%) compared with raw milk (23.5%). There are several reasons behind the low isolation of *Listeria* from the cheese. The first reason could be attributed to the high concentration of salt and the low acidity and moisture content of cheeses for inhibiting *Listeria* growth. Bacteriocins produced by other bacterial species like *Enterococcus* and *Lactobacillus* can also suppress *Listeria* growth in cheese [32].

Regarding chicken meat, raw beef and beef sausage, our results showed the highest incidence (17.4%) of *L. monocytogenes* of samples containing *Listeria* spp. These values are in agreement with studies performed by [33],[34] who found *L. monocytogenes* prevalence 20.4% and 21.6% respectively. However, the occurrence of *L. monocytogenes* in chicken meat according to [23] study was as low as 8%. The variation in prevalence of *L. monocytogenes* in samples might be due to the differences in holding time, processing ways of the food before sale, as well as this microorganism is able to survive in low temperature [35] and tolerate cold stress [36].

Unlike most previous studies, no *L. ivanovii* and *L. seeligeri* were discovered in all tested samples. *L. seeligeri* considered to be non-pathogenic such other species of *Listeria* (*L. welshimeri*, *L. murrayi* and *L. grayi*). *L. monocytogenes* is pathogenic for humans and animals, and *L. ivanovii* is mainly pathogenic for animals, primarily sheep.

In conclusion, considering the risk factors associated with contamination of raw milk and meat products by *L. monocytogenes*, which includes, inadequate frequency of cleaning the exercise area, protection against the contamination of milk and dairy products with *L. monocytogenes* in the production process must be focused on control in all production stages, starting from the primary production of milk on the farm, in dairy plant, during storage and distribution. Although the conventional method used in the present study allowed a reliable identification of *L. monocytogenes* and other *Listeria* spp. molecular work is needed which might help to investigate the importance of *Listeria* spp. as causative agents of Foodborne disease. Therefore, further research on molecular identification of *Listeria* and its virulence genes will be carried out in order to provide a better background of contamination rate and routes of transmission for this bacterium.

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