

SEASONAL CHANGES IN THE OCCURRENCE OF *LISTERIA MONOCYTOGENES* IN DUHOK PROVINCEAazd M. T. Al-Brefkani ^{a,*} and Ismaeil M. A. Mammani^b^aCollege of Medicine, University of Duhok, Kurdistan region-Iraq- azad.tahir@dpu.edu.krd^bCollege of Health Science, University of Duhok, Kurdistan region-Iraq- ismaeil.mohammed@uod.ac**Received: Nov. 2018 / Accepted: Dec., 2018 / Published: Mar., 2019**<https://doi.org/10.25271/sjuoz.2019.7.1.549>**ABSTRACT:**

Listeria monocytogenes are Gram-positive bacilli cause listeriosis, associated with high rates of hospitalization and mortality. A total of 1362 samples were investigated included 647 clinical samples and 715 from food. This study investigated the differences in the seasonal occurrence of *L. monocytogenes* in Kurdistan region-Iraq. *L. monocytogenes* was found in 48 samples (3.52%) out of 1362 samples. There were 7/642(1.09%) human clinical samples diagnosed with cold and moderate weather (November, December 2016, and January, February, April 2017). While 41/715(5.73%) of *L. monocytogenes* isolates were found among food samples, meat samples (n=37) and dairy samples (n=4). A total of 37/476 (7.77%) of the *L. monocytogenes* isolates in meat samples were detected during warm weather (from July through October 2016). In addition, *L. monocytogenes* in dairy products was found to be by 4/239 (1.68%), were isolated during the moderate season (October, November 2016, and April, May 2017). The study found that the incidence of meat contamination by *L. monocytogenes* increase significantly during the warm season in comparison with other seasons. Furthermore, the cases of human Listeriosis caused correlated well with the seasonal levels of *L. monocytogenes* found in dairy products. A statistically significant difference in the occurrence of *L. monocytogenes* isolates and seasons were identified in this study (P value= <0.05).

KEYWORDS: *Listeria monocytogenes*, Listeriosis, Seasonal Changes, Clinical Specimens.**1. INTRODUCTION**

Listeria monocytogenes is a gram positive, mesophilic, facultative anaerobic bacterium. It is implicated in numerous food poisoning cases and foodborne infection outbreaks. This bacterium is unique because of its high mortality rate (20%- 30%) (Mead et al., 2000). The majority (99%) of listeriosis cases are assumed to be through consumption of contaminated food (Swaminathan and Gerner-Smidt, 2007). Immuno-compromised individuals, newborns, pregnant women and the elderly are especially vulnerable to Listeriosis due to *L. monocytogenes* infection (Farber and Peterkin, 1991). Listeriosis can appear as a moderate non-invasive gastrointestinal disease or invasive illness as meningitis or septicemia (Vázquez-Boland et al., 2001). Spontaneous third trimester abortions, encephalitis, meningitis and septicemia are the most severe clinical manifestations of invasive Listeriosis. The more common clinical manifestations of Listeriosis include mild influenza-like infection, occasionally combined with gastroenteritis (McLauchlin et al., 2004). Up to date, 17 species related to genus *Listeria* have been established which includes: *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, *L. grayi*, *L. marthii*, *L. rocourtiae*, *L. fleischmannii*, *L. weihenstephanensis*, and those recently added *L. floridensis*, *L. aquatica*, *L. cornellensis*, *L. riparia*, *L. grandensis*, *L. booriae*, and *L. newyorkensis* (Weller et al., 2015). *Listeria monocytogenes* was confirmed as a primarily pathogenic to human and causing listeriosis. Whilst, *L. ivanovii* and *L. seeligeri* have also been associated with human infection in very rare cases (Guillet et al., 2010; Poulsen and Czuprynski, 2013). Seasonal distribution of this bacterium is a great concern and have been documented by numerous researchers (Jones et al., 1990; Strauch, 1991; MacGowan et al., 1994; Hitchens, 1995; Dumontet et al., 1997). The study is aimed to investigate the seasonal variations in the occurrence of *L. monocytogenes* in

Duhok province-Kurdistan region. Seasons are defined as follows: Warm season occurs in the months of (June, July, August, and September); the moderate season is in the months of (October, March, April, and May); while cold season happens in (November, December, January, and February).

2. MATERIALS AND METHODES**2.1 Sample Source**

A total of 1362 samples comprising of patient derived (n=647) and food derived (n=715) samples were evaluated in the present study. Clinical samples included pregnant women who have had the history of obstetrical complications (200 High vaginal swabs and 200 Urine samples), and blood samples were collected from 247 immuno-compromised patients whose ages ranged from 1 day to 85 years old. These immune-compromised patients suffered from different clinical problems; 101 with renal failure patients, 83 septicemia cases, 44 premature infants, 15 meningitis cases, and finally 4 heart failure cases were included in this analysis. On the other hand, 715 food samples were investigated in this study. Among them, 476 were meat samples; 309 chicken meat and 167 red meat. While, 239 samples were dairy products. Among them, 118 samples were from locally produced sheep raw milk and 121 samples were from locally prepared white soft cheese. Samples were taken at random during each season.

2.2 Bacteriological Isolation

This bacterium was isolated according to the procedure described by ISO 11290-1:1997 (Aznar and Solis, 2006). Double enrichment broth (Half Fraser & Fraser) in addition to Harlequin™ *Listeria* Chromogenic agar was used to differentiate *L. monocytogenes* from other *Listeria* spp.

2.3 Biochemical Tests

Microgen *Listeria*-ID microwell test strip which contains 11 dried carbohydrate substrates which recommended in

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international standard methods (Hitchins, 2003) and the hemolysin reaction were performed in well number 12 (Dominguez Rodriguez et al., 1986).

2.4 Molecular Investigation

Isolation of genomic DNA from bacterial colonies was carried out using the boiling method according to the protocol described by (Adzitey et al., 2013; Dashti et al., 2009). 16S rRNA gene primer was described by (Park et al., 2016). The amplification reactions were accomplished in 25µl volume and 100 bp DNA ladder (GeNet Bio, South Korea) was used as a size marker (M) in all gels. The amplified DNA bands were separated by using agarose gel electrophoresis as designated by (Park et al., 2016).

2.5 Statistical Analysis

Chi-Square Analysis was used to show the significant differences among different seasons in the occurrence of *L. monocytogenes*.

3. RESULTS

The first molecular diagnostic step to confirm *L. monocytogenes* isolates was targeting the preserved regions of this bacterium 16SrRNA gene (Figure 1).

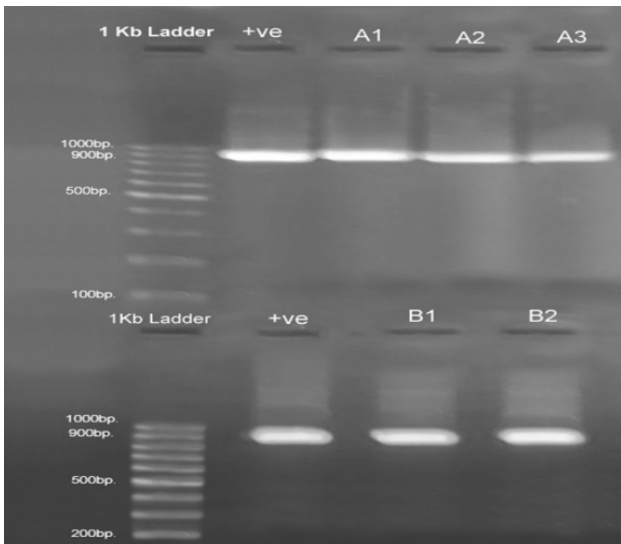


Figure 1: An example of 16SrRNA positive gene of *L. monocytogenes* isolated from food sources and clinical cases.

1kb molecular weight ladder was used in this PCR reaction. Lane 2 is positive control, (A1-A3) are 16SrRNA (938bp) positive gene of *L. monocytogenes* isolated from food sources. (B1-B2) are 16SrRNA positive gene of *L. monocytogenes* isolated from clinical cases.

The seasonal occurrence of *L. monocytogenes* isolates from food and clinical samples were analyzed in this study. *L. monocytogenes* was found in 48/1362 (3.52%) of samples. the vast majority of positive samples were diagnosed during warm weather 30/48 (62.50%). During the moderate season, 11/48 (22.92%) of the samples were found to be contaminated with *L. monocytogenes*., while only 7/48(14.58%) of positive samples were detected in cold season. A significant correlation was detected between isolated *L. monocytogenes* and seasons (P value= 0.00001) (Table 1, Figure 2).

Table 1: *Listeria monocytogenes* isolates derived from food and patient sources during cold, moderate and warm seasons

Sample \ Seasons	<i>Listeria</i> isolated from Meat Products	<i>Listeria</i> isolated from Dairy Products	<i>Listeria</i> isolated from Patients	Total Isolates
Cold Season	0 (0.0%)	2(4.17%)	5(10.41%)	7(14.58%)
Moderate Season	7(14.58%)	2(4.17%)	2(4.17%)	11(22.92%)
Warm Season	30(62.5%)	0(0.0%)	0(0.0%)	30(62.50%)
Total	37(77.08%)	4(8.34%)	7(14.58%)	48 (100%)

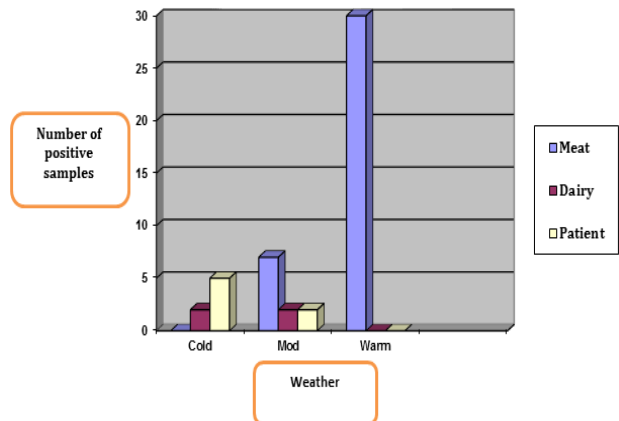


Figure 2: Shows *Listeria monocytogenes* isolated from food and patient sources during cold, moderate and warm seasons

3.1 Pregnant Women

The highest incidence of Listeriosis that occurred among pregnant women was seen during the November 2/82(2.43%), By contrast, during the months December and January there were fewer cases of Listeriosis, by 1/154(0.64%) and 1/113(0.88%) respectively. No positive samples were detected in May and June. There wasn't statistically significant difference in the occurrence of cases of Listeriosis among pregnant women during the aforementioned months (P-value=0.31) (Table 2).

Table 2: Distribution of listeriosis cases among pregnant women according to seasons.

Date \ No., % of samples	Positive (No. &%)	Negative (No. &%)	Total
May-2016	0 (0.0%)	26 (100%)	26
June- 2016	0 (0.0%)	25 (100%)	25
November-2016	2 (2.43%)	80 (97.57%)	82
December-2016	1 (0.64%)	153 (99.36%)	154
January-2017	1(0.88%)	112 (99.12%)	113
Total	4 (1.0%)	396 (99.00%)	400 (100%)

3.2 Other Immunocompromised Patients

Regards other immunocompromised patients (Renal failures, septicemia, premature infants and meningitis patients) dispersal of Listeriosis cases regarding the seasons is shown in (Table 3). Out of three positive cases, 1/19(5.26%) was found in February

2017, whereas the other 2/130(1.53%) cases were diagnosed in April-2017. No significant correlation was found between seasons and *Listeria* cases (P-value= 0.31), (Table 3).

Table 3: Distribution of Listeriosis cases among Immuno-Compromised individuals according to seasons.

Date	Positive (No. & %)	Negative (No. & %)	Total
January-2017	0(0.0%)	18 (100%)	18
February-2017	1(5.26%)	18 (94.74%)	19
March-2017	0 (0.0%)	39 (100%)	39
April-2017	2(1.53%)	128(98.47)	130
June-2017	0(0.0%)	41(100%)	41
Total	3 (1.21%)	244(98.79%)	247

3.3 Meat

The highest rate of *L. monocytogenes* isolates was detected during August 2016 27/208 (12.98%). In addition, 2.77%, 3.23%, and 5.83% of isolates were demonstrated during July, September, and October respectively. There statistically significant relationship was found between the date of isolation and *L. monocytogenes* contamination in meat products (P-value= 0.00079), (Table 4).

Table 4: Distribution of *Listeria monocytogenes* contamination among meat products according to the season of isolation.

Date	Positive (No. & %)	Negative (No. & %)	Total
July-2016	1(2.77%)	35 (97.23%)	36
August-2016	27(12.98%)	181(87.02%)	208
September-2016	2(3.23%)	60 (96.77%)	62
October-2016	7 (5.83%)	113(94.17%)	120
November-2016	0 (0.0%)	50(100%)	50
Total	37 (7.7%)	439 (2.23%)	476

3.4 Dairy Products

The sampling was started on 31st July 2016 and terminated on 10th-MAY-2017. No isolates of *L. monocytogenes* were obtained during the months of July and August 2016 (Table 5). A single isolate of *L. monocytogenes* was isolated in October and in November 2016. A single isolate of *L. monocytogenes* was isolated in April and May 2017. A statistically significant relationship was observed between *L. monocytogenes* contamination and month of sampling (P-value=0.0076).

Table 5: Distribution of *Listeria monocytogenes* contamination according to date of sampling among dairy products

Date	Positive (No. & %)	Negative (No. & %)	Total examined samples
JUL-2016	0(0.00%)	3 (100%)	3
AUG-2016	0(0.00%)	1(100%)	1
OCT-2016	1 (4.76%)	20 (95.24%)	21
NOV-2016	1 (11.11%)	8(88.89%)	9
APR-2017	1 (1.00%)	99 (99.00%)	100
MAY-2017	1(0.95%)	104(99.05%)	105
Total	4 (1.68%)	235 (98.32%)	239

3. DISCUSSION

This investigation looked at the Seasonal distribution of *L. monocytogenes* in Duhok province. The seasonal differences of *L. monocytogenes* in Kurdistan region have not been previously reported. Cases involving human Listeriosis occurred during cold weather and during the natural grazing season. The results obtained in our study differed from those obtained by the European Food Safety Authority study (EFSA, 2015). The EFSA study reported that human Listeriosis increased slightly during the warm season as compared with the other seasons of the year (Authority and Control, 2016). In addition, A study carried out in the United States obtained results that were disagree to those obtained in our study, where the vast majority of listeriosis cases were detected during August and September (Voetsch et al., 2007). The differing results obtained in this investigation could be due to differences in sample size variation and differentiation of the temperature between countries. In the present study, an increased number of contaminated meats with *Listeria* contamination were obtained during warm weather. An elevated percentage (12.98%) was detected in August. Our results are similar to the findings of other earlier investigations (Rhoades et al., 2009; Elmali et al., 2015; Alewy et al., 2016). Earlier investigations failed to detect a seasonal pattern of *L. monocytogenes* conducted by (Wilson, 1995; GOMBAS et al., 2003). Regarding dairy products, a statistical significant relationship between *L.monocytogenes* isolates and the season of isolation were detected (P value= 0.0076), this study found that contamination with *L. monocytogenes* is more prevalent in two seasons only. Of four positive samples of *L. monocytogenes* that were isolated, 2 were isolated in spring and 2 in winter. Perhaps the unequal distribution of *L. monocytogenes* can be linked to: a) quality of feed given to animals, and b) variations in the global environment. Silage is commonly used by the Kurdish Farmer as animal feed. The percentage of contamination of silage with *L. monocytogenes* can be linked to how the silage is stored and the procedures used to prepare the silage. The rate of *L. monocytogenes* contaminated was probably due to the high rate of silage feeding in that season (Boerlin et al., 2003). The study of Boerlin proved that poor quality of silage is the main reason for an elevated incidence of *listeriosis* infections among ruminants. The lower incidence of contamination with *L. monocytogenes* in summer and in spring is most probably due to the animals grazing out in the fields on natural feed sources rather than silage (Fenlon et al., 1996). The high rate of raw milk contaminated with *L. monocytogenes* in winter season was confirmed by an earlier study (Meyer-Broseta et al., 2003). In conclusion, the study found that the incidence of meat

contamination by *L. monocytogenes* increase significantly during the warm season in comparison with other seasons. Furthermore, the cases of human Listeriosis caused correlated well with the seasonal levels of *L. monocytogenes* found in dairy products. A statistically significant difference in the occurrence of *L. monocytogenes* isolates and seasons were identified in this study (P value= <0.05).

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