

Seasonal effect on *L. monocytogenes* prevalence in meat and dairy products assessed by VIDAS LMO2 and ISO 11290:1 methods

¹*Kevenk, T. O. and ²Koluman, A.

¹Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Aksaray University, TR 68000 Aksaray, Turkey

²Department of Biomedical Engineering, Faculty of Technology, Pamukkale University, TR 20160 Denizli, Turkey

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Abstract

In the present work, the seasonal distribution of *L. monocytogenes* in frequently consumed foods in the Aksaray region, an important transition point, was investigated by cultural and automated methods (ISO 11290 and VIDAS LMO2). For this purpose, a total of 800 food samples (100 samples of each white, kashar, cream, and Tulum cheeses, and beef, lamb, chicken, and turkey meats) were analysed. *Listeria* spp. were detected in 64 (8%) samples, and 177 suspected *Listeria* colonies were isolated. Of the 177 suspected colonies, 71 were identified as *L. monocytogenes* by the ISO 11290 and VIDAS LMO2 methods. The pathogen was detected from samples purchased during winter, spring, summer, and autumn at the rates of 3.7, 3, 26, and 3.6%, respectively; the highest isolation rate was found in summer, while the lowest isolation rate found in spring. Although the contamination of *L. monocytogenes* was found at the highest rate in summer, it has been revealed that there was a risk of listeriosis, which was not low, throughout the entire year. We believe that compliance with standards such as HACCP, ISO 22000, or GMP will be crucial in reducing the risk of listeriosis.

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Introduction

Listeriosis, which is caused by *Listeria monocytogenes*, a Gram-positive, ubiquitous, facultative anaerobe, psychrotroph, and intracellular agent, is known as a significant food-borne disease today. Presently, the genus *Listeria* has 21 identified species and six subspecies. However, only two of these, *L. monocytogenes* and *L. innocua*, are pathogenic (Kaszoni-Rückerl *et al.*, 2020). Meningitis, encephalitis, miscarriage, premature birth, septicaemia, diarrhoea, and fever are the severe symptoms of food-borne listeriosis. A high mortality rate (20 - 30%) has also been reported as a serious consequence of listeriosis. It has been stated that the disease may be caused by the consumption of various raw and processed foods such as unpasteurised milk, soft or semi-soft cheeses, ready-to-eat foods, and unwashed fruits and vegetables (Cossart and Mengaud, 1989; Gandhi and Chikindas, 2007; Buchanan *et al.*, 2017; Forauer *et al.*, 2021).

In the past two decades, the numbers of outbreaks reported globally have seriously increased

by means of more effective detection of *L. monocytogenes* and improvements in diagnostic methods and technology. EFSA reported 1,763 human listeriosis cases in the EU associated with crustaceans, shellfish, molluscs and products, cheeses, meat and meat products, pork meat and products, and vegetables and juices (ready-to-eat salad) in 2013 (EFSA, 2015; ECDC, 2016). In the USA, the Centers for Disease Control and Prevention (CDC) announced that 568, 621, 582, 646, and 675 cases of listeriosis were detected in 2010, 2011, 2012, 2013, and 2014, respectively (CDC, 2012; 2013; 2014; 2015; 2016). Similarly, in Turkey, numerous studies have been conducted on the prevalence of *L. monocytogenes* in foods. Elmali *et al.* (2015) isolated *L. monocytogenes* in 47.5% of broiler wings samples. Ayaz *et al.* (2018) detected the pathogen in 3.4 and 2.5% of cattle and sheep meat samples, respectively. Terzi *et al.* (2015) investigated ready-to-eat foods, and *L. monocytogenes* was determined in 16 (16%) of their samples. Kevenk and Terzi Gulel (2016) investigated milk and dairy products in the Black Sea region of Turkey, and found that 12% of milk samples

*Corresponding author.

Email: tahsinonurkevenk@aksaray.edu.tr

and 20% of dairy products were contaminated with *L. monocytogenes*.

Due to the changes in seasonal patterns and global food consumption habits, food-borne outbreaks have significantly increased. According to the European CDC, confirmed listeriosis cases showed a seasonal distribution between 2010 and 2013, and the highest listeriosis cases were announced in the EU from June to September (summer months) (ECDC, 2016).

Milk and dairy products have been reported as the primary calcium source in the US diet. In addition, dairy foods, turkey, chicken, beef, and lamb contain generous quantities of other essential nutrients such as minerals, vitamins, proteins, and essential amino acids (Huth *et al.*, 2006). According to Turkish Statistical Institute (TUIK), around 1,071,903, 733,189, 490,162, 23,503, 2,136,263, 58,212, and 1,201,500 tone milk, yogurt, white cheese, other cheeses, chicken, turkey, and beef meat were produced in Turkey in 2020, respectively (TUIK, 2019; 2020a; 2020b).

Aksaray, located in the central part of Turkey, is a stopover region for south, north, and west coasts of the country. For this reason, many tourists can easily have the opportunity to meet local delicacies during travel breaks, and this situation may lead to the risk of possible food poisoning cases. In addition, no comprehensive prevalence studies were done in the region which hosts heavy tourist transitions throughout the year. According to the data of the General Directorate of Highways, it has been reported that the daily traffic volume of Aksaray highway is between 20,000 and 50,000 vehicles in 2020 (KGM, 2021), and this volume is increasing day by day through new highways.

The objectives of the present work was therefore (1) to determine seasonal effect on *L. monocytogenes* prevalence in the most preferred meat and dairy products sold in Aksaray, and (2) to understand the effectiveness of VIDAS and ISO 11290:1 methods in analysing natural food microflora.

Materials and methods

The present work aimed to reveal the seasonal prevalence of *L. monocytogenes* in the Aksaray region. For this purpose, both the classical culture technique recommended by ISO 11290:1 and the fast

and reliable VIDAS method were used in meat and dairy products.

Sampling of meat and dairy products

A total of 800 meat and dairy products were purchased; 400 meat products (100 of each beef, chicken, lamb, and turkey meats) and 400 dairy products (100 of each white, kashar, cream, and hard Tulum cheese). The samples were monthly purchased from local bazaars, roadside restaurants, and markets from Aksaray region between April 2020 - March 2021. The samples were collected from 18 different sale points and dealers. Then, the samples were immediately transported to the laboratory under cold chain (4°C), and immediately analysed.

Isolation and purification of L. monocytogenes with ISO 11209:1 and VIDAS methods

Isolation was performed following the ISO 11290:1 method. Identification and confirmation were performed using API *Listeria* test strips (bioMerieux) (ISO, 2017). Briefly, 25 g of sample was aseptically weighed and placed into sterile sample bag. Next, 225 mL of Half Fraser Broth was added (LabM, LAB211) into the bag and homogenised using a stomacher (Interscience Bagmixer 400) for 1 min. Samples were incubated for pre-enrichment at 30°C for 24 h. Later, Fraser Broth was used for selective enrichment for another 24 h (LabM, LAB164). *Listeria* Isolation Medium (Oxford, LabM, LAB122) and specific supplements were used. Suspected typical colonies were streaked on to Tryptic Soy Agar (LabM, LAB011) with 0.6% yeast extract (Oxoid LP0021B), and incubated at 37°C for 24 h. Biochemical confirmation of suspected strains was performed on API *Listeria* test strips, and the results were evaluated in comparison with the guide provided by the manufacturer.

The VIDAS LMO2 kits were used for detection of *L. monocytogenes* as well. After the selective enrichment step, 500 µL of sample and Fraser Broth mixture were pipetted into the LMO2 kit well. Then, strips were placed in the VIDAS device, and results were obtained in approximately 70 min. In the end, positive samples were confirmed using API *Listeria* biochemical identification tests (Johnson, 2013). Isolation and purification steps of *L. monocytogenes* is shown in Figure 1.

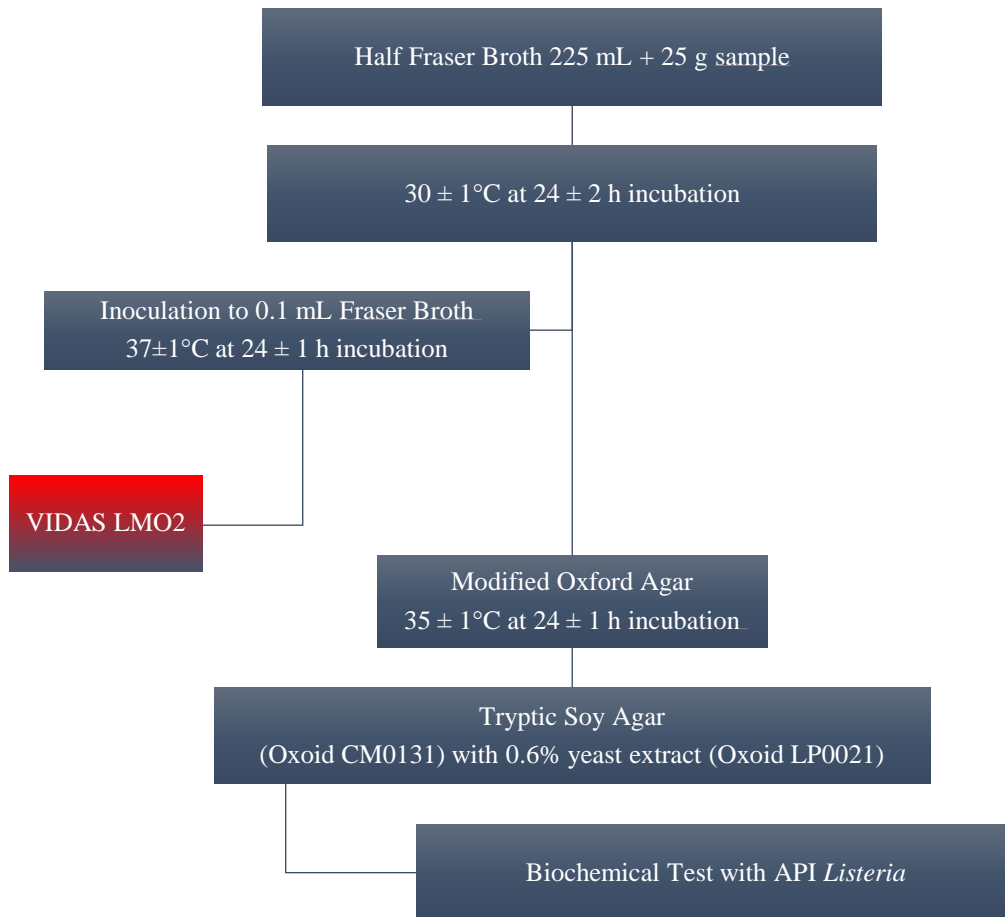


Figure 1. Isolation and purification steps of *L. monocytogenes*.

Statistical analyses

Statistical analyses were performed using SPSS v26 (2019, IBM) software. The Kruskal-Wallis H test (non-parametric) was used for identifying compatibility between results.

Results and discussion

In the present work, a total of 800 samples (400 meat and 400 dairy products) were analysed seasonally for the presence of *L. monocytogenes*. The results are shown in Table 1.

Table 1. Prevalence of *L. monocytogenes* in meat and dairy products.

Sample	n	ISO 11290:1 + API		VIDAS LMO2 + API	
		No. of <i>Listeria</i> spp. positive samples	Suspected <i>Listeria</i> spp. colonies	<i>L. monocytogenes</i> confirmed colonies	<i>L. monocytogenes</i> confirmed colonies
White cheese	100	5 (5%)	21	18	16
Kashar cheese	100	3 (3%)	11	7	7
Cream cheese	100	3 (3%)	8	8	7
Hard Tulum cheese	100	6 (6%)	23	11	11
Beef meat	100	19 (19%)	40	11	10
Lamb meat	100	6 (6%)	18	6	6
Chicken meat	100	13 (13%)	32	5	5
Turkey meat	100	9 (9%)	24	5	4
Total	800	64 (8%)	177	71	66

Listeria spp. were detected in 64/800 (8%) samples, and 177 *Listeria* spp. colonies were isolated. Based on identification tests, 71 isolates were confirmed as *L. monocytogenes* by ISO 11290:1 and API *Listeria*, but only 66 isolates were confirmed as *L. monocytogenes* by VIDAS LMO2 and API *Listeria*. Between the two methods, 3% of isolation difference was determined. The fact that a smaller number of *L. monocytogenes* was isolated with the VIDAS LMO2 technique demonstrated once again the sensitivity of classical culture technique (ISO method). In addition, although the contamination rate in dairy products was found lower than in meat products, this ratio should be seriously taken into account since the dairy products are in the ready-to-eat food category, readily available.

For dairy products, *L. monocytogenes* was detected in 5% of white cheese samples. Kevenk and Terzi Gulel (2016) detected the pathogen at 5% in white cheese samples in Samsun/Turkey. Besides, Kahraman *et al.* (2010) detected the pathogen in 4.8% white cheese samples as well. Aksoy *et al.* (2018) detected the pathogen at 4% in same sample type. These results showed similarities with our study. On the other hand, Arslan and Özdemir (2008) and Almeida *et al.* (2013) isolated *L. monocytogenes* in cheese samples, and found 9.2 and 13.6%, respectively. *L. monocytogenes* is known as halotolerant and acid-resistant bacteria (Ferreira *et al.*, 2014). In our study, 3% of kashar cheese samples were contaminated with *L. monocytogenes*. Kahraman *et al.* (2010), Cagri-Mehmetoglu *et al.* (2011), and Kevenk and Terzi Gulel (2016) isolated the pathogen at 1.7, 2.6, and 0%, respectively. *L. monocytogenes* incidence in cream cheeses was found at 3% in the present work. Di Pinto *et al.* (2010), Martinez-Rios and Dalgaard (2018), and Churchill *et al.* (2019) reported that the prevalence of *L. monocytogenes* was 1.9, 2.2, and 2.4%, respectively. Our research found the highest contamination rate in Tulum cheese (bryndza), which has a very salty nature. Cokal *et al.* (2012) and Karadal and Yildirim (2014) detected *L. monocytogenes* at 1 and 5%, respectively. In the studies mentioned before, the amount of salt and lactic acid, differences in the production area, human factors, farm applications (feed and silage quality) and isolation, and identification procedures can be cited among the reasons for the differences between the results. Moreover, pasteurisation conditions and

cross-contamination may also affect on hygienic quality of samples. These factors can be considered as an explanation for the differences observed between the research findings above.

For meat products, *L. monocytogenes* was detected in beef, lamb, chicken, and turkey meats at 11, 6, 5, and 5%, respectively. Islam *et al.* (2016) isolated *L. monocytogenes* in beef and chicken meats at 8 and 16%, respectively. However, their total sample number was relatively low; 12 beef and 12 chicken samples. The reason for the high percentage of findings can be attributed to the low sample number. At the same time, hygienic environmental conditions may also have contributed to the high rate of results. In another study, Wieczorek *et al.* (2012) investigated 417 beef samples in Poland, and reported that 19.4% of them were contaminated with *L. monocytogenes*. Our study showed lower isolation rates than the study above. This situation may relate to the hygienic quality of our samples, storage conditions, and stability of our method. Elmali *et al.* (2015) investigated 120 chicken wing samples for the presence of *L. monocytogenes* in a whole year period. Based on their results, 37.5% of the samples were contaminated with the pathogen. In comparison with our study, the higher findings may be linked with the microbial load of poultry houses, hygienic conditions of the slaughterhouses, personal hygiene, and high levels of cross-contamination. In a study conducted by Bohaychuk *et al.* (2006) in Canada, it was reported that 3% (3/100) of turkey samples were contaminated with *L. monocytogenes*. In other studies, Nørrung *et al.* (1999) in Denmark, Uyttendaele *et al.* (1999) in Belgium, and Vitas and Garcia-Jalon (2004) in Spain found that the *L. monocytogenes* detection rates in turkey meat were 5, 6.7, and 4.9%, respectively. As can be seen, these results are lower than those reported in the present work. That is why it may be crucial to raise the hygienic quality of food processing procedures and transportation conditions from producers through the market. In this way, excessive reproduction of pathogens can be prevented.

The seasonal distribution of *L. monocytogenes* was also investigated in the present work, and it was observed that the prevalence of *L. monocytogenes* dramatically increased in warmer months (June, July, August). However, due to its psychrotrophic character, it was also isolated in colder months (Table 2).

Table 2. Monthly distribution of purchased samples and presence of *Listeria*. spp. and *L. monocytogenes* isolates.

Sample	2020												2021			Total										
	Apr		May		June		July		Aug		Sept		Oct		Nov		Dec		Jan		Feb		Mar			
	TS (PS)	C/I	TS (PS)	C/I	TS (PS)	C/I	TS (PS)	C/I	TS (PS)	C/I	TS (PS)	C/I	TS (PS)	C/I	TS (PS)		C/I	TS (PS)	C/I	TS (PS)	C/I	TS (PS)	C/I	TS (PS)	C/I	
White cheese	8 (0)	0	8 (1)	5/4	8 (2)	7/7	8 (1)	4/3	8 (0)	0	8 (0)	0	8 (0)	0	8 (0)	0	9 (0)	0	9 (1)	5/4	9 (0)	0	9 (0)	0	100 (5)	21/18
Kashar cheese	8 (0)	0	8 (1)	5/3	8 (1)	3/2	8 (1)	3/2	8 (0)	0	8 (0)	0	8 (0)	0	8 (0)	0	9 (0)	0	9 (0)	0	9 (0)	0	9 (0)	0	100 (3)	11/7
Cream cheese	8 (0)	0	8 (1)	3/3	8 (1)	2/2	8 (1)	3/3	8 (0)	0	8 (0)	0	8 (0)	0	8 (0)	0	9 (0)	0	9 (0)	0	9 (0)	0	9 (0)	0	100 (3)	8/8
Hard Tulum cheese	8 (0)	0	8 (1)	4/2	8 (1)	5/2	8 (1)	3/3	8 (1)	4/3	8 (1)	0	8 (0)	0	8 (0)	0	9 (0)	0	9 (1)	3/1	9 (0)	0	9 (0)	0	100 (6)	23/11
Beef	8 (2)	4/2	8 (3)	6/2	8 (2)	5/2	8 (3)	5/1	8 (1)	2/0	8 (1)	2/0	8 (1)	2/0	8 (1)	3/1	9 (1)	2/0	9 (1)	2/1	9 (1)	3/1	9 (1)	2/0	100 (19)	40/11
Lamb	8 (1)	4/1	8 (1)	2/1	8 (1)	4/2	8 (1)	4/0	8 (1)	2/1	8 (1)	0	8 (0)	0	8 (0)	0	9 (0)	0	9 (0)	0	9 (0)	0	9 (0)	0	100 (6)	18/6
Chicken	8 (1)	2/0	8 (1)	4/1	8 (2)	4/2	8 (1)	2/0	8 (1)	2/0	8 (1)	4/1	8 (1)	2/0	8 (1)	2/0	9 (1)	2/0	9 (1)	2/0	9 (1)	2/0	9 (1)	2/0	100 (13)	32/5
Turkey	8 (0)	0	8 (1)	4/0	8 (2)	4/1	8 (1)	4/1	8 (1)	2/0	8 (1)	2/1	8 (1)	2/1	8 (0)	2/0	9 (0)	2/1	9 (0)	0	9 (0)	0	9 (0)	0	100 (9)	24/5
Total	64 (4)	10/3	64 (6)	18/3	64 (11)	33/17	64 (12)	28/13	64 (10)	12/4	64 (4)	8/2	64 (3)	6/1	72 (3)	6/1	72 (2)	6/1	72 (4)	12/6	72 (2)	5/1	72 (2)	4/0	800 (64)	177/71

TS: total purchased sample; PS: number of *Listeria* spp. positive sample; C: number of *Listeria* spp. colonies; and I: number of identified *L. monocytogenes* isolates.

Our results were also evaluated statistically, and seasonal differences in our isolation rates were found to be significant ($p < 0.05$). *L. monocytogenes* was detected at 3.7, 3, 26, and 3.6% from the purchased samples in winter, spring, summer, and autumn, respectively. Considering the annual average temperature data of Aksaray, it can be seen that the summer season was quite hot (Table 3). This situation may be the main reason why *L. monocytogenes* was

isolated with the highest percentage in the summer season in the present work. In line with our work, according to ECDC between the years 2010 – 2013, the confirmed listeriosis cases in warm months such as June, July, and August were higher than other months in the EU (ECDC, 2016). For this reason, the consequences of failures that may occur during the transportation and storage of foods in warm months may be more severe.

Table 3. Monthly temperature (°C) mean of Aksaray region (Turkish State Meteorological Service).

Temperature	2020						2021					
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Mean	11.5	16.2	20.2	23.5	23.2	18.7	13.3	7.1	2.6	0.5	2	6.4
Maximum	18	23	27.1	30.7	30.7	26.7	21	13.7	7.7	5.4	7.5	12.6
Minimum	5.5	9.6	13.1	16.2	15.9	11.3	6.8	1.9	-1.4	-3.6	-2.2	1.3

Conclusion

Listeriosis is a significant foodborne disease with its variable host dynamics and increased mortality. In the present work, it was evident that *L. monocytogenes* cases were increasing in warmer months. However, seasonal distribution was not the only reason for the increased prevalence; not applying or practicing hygienic and safe food measures could also be the reason. *L. monocytogenes* infections, even under the influence of climate change, can be predicted by food safety measures and hygienic practices.

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