



Presence and Prevalence of *Listeria* Species in the Inci Kefali (*Chalcalburnus tarichi*, Pallas 1811)*

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ABSTRACT

Lake Van is not a very convenient environment for life of both sea and freshwater fish due to its high alkaline (pH 9.8) and salty (0.21%) water and only pearl mullet (*Chalcalburnus tarichi*, Pallas 1811) are present in the lake. In this study, the presence and prevalence of *Listeria* species in samples of the pearl mullet (*Chalcalburnus tarichi*, Pallas 1811) living as an endemic species in Lake Van and taken from the market a total of 160 times for 12 months were examined. The method recommended by the United States Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) was used for the isolation and identification of *Listeria* species. Among the pearl mullet samples examined, *Listeria* spp. were isolated in 20 (12.50%) and of them *L. innocua* in 10 (6.25%) and *L. welshimeri* in 8 (5.00%) and *L. ivanovii* in 2 (1.25%) were identified. In this study, the presence of *Listeria* species in pearl mullet samples examined according to the seasons was revealed for the first time. Failure to identification of *L. monocytogenes* in any of the samples that is an important pathogen for humans, was assessed as an indicator that these fish did not constitute a public health risk in terms of listeriosis. As a result, *Listeria* spp. and other pathogenic microorganisms not to pose a risk to public health and in order to prevent the deterioration of fishes and reduce the economic losses, the transportation, storage and marketing of the caught fishes should be done under hygienic and cold conditions.

Keywords: Fish, Inci kefali (*Chalcalburnus tarichi*), *Listeria* spp.

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İnci Kefalinde (*Chalcalburnus tarichi*, Pallas 1811) *Listeria* Türlerinin Varlığı ve Yaygınlığı

Van Gölü yüksek derecede alkali (pH 9.8) ve tuzlu (%0.21) suyu ile hem deniz hem de tatlı su balıklarının yaşamı için çok elverişli bir ortam değildir ve gölde sadece inci kefali (*Chalcalburnus tarichi*, Pallas 1811) bulunmaktadır. Bu çalışmada, Van Gölü'nde endemik bir tür olarak yaşayan ve 12 ay süreyle toplam 160 defa piyasadan alınan inci kefali örneklerinde *Listeria* türlerinin varlığı ve yaygınlığı incelenmiştir. *Listeria* türlerinin izolasyon ve identifikasyonunda United States Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) tarafından önerilen metot kullanılmıştır. İncelenen inci kefali örneklerinin 20 (%12.50)'sinde *Listeria* spp. izole edilmiş ve bunların 10 (%6.25)'unda *L. innocua*, 8 (%5.00)'inde *L. welshimeri* ve 2 (%1.25)'sinde de *L. ivanovii* identifiye edilmiştir. Bu araştırma ile mevsimlere göre incelenen inci kefali örneklerinde *Listeria* türlerinin varlığı ilk defa ortaya konmuştur. Hiçbir örnekte insanlar için önemli bir patojen olan *L. monocytogenes*'in belirlenmemesi bu balıkların listeriosis yönünden bir halk sağlığı riski oluşturmadığının göstergesi olarak değerlendirilmiştir. Sonuç olarak, gerek *Listeria* spp. (özellikle *L. monocytogenes*) ve diğer patojen mikroorganizmaların halk sağlığı riski oluşturmaması ve gerekse balıkların bozulmasının önlenerek ekonomik kayıpların önüne geçilmesi için, avlanan balıkların nakil, depolama ve pazarlamasının hijyenik şartlarda ve soğukta yapılmasının gerekli olduğu kanaatine varılmıştır.

Anahtar Kelimeler: Balık, İnci kefali (*Chalcalburnus tarichi*), *Listeria* spp.

INTRODUCTION

In the fish production in inland waters, the pearl mullet (*Chalcalburnus tarichi*, Pallas 1811) (Kuru 1985) belonging to *Cyprinidae* family takes the second place after carps.

Only pearl mullet inhabits in Lake Van (pH 9.8 and salinity of 0.21%), which is not a suitable environment for sea and freshwater fish (Sarı 2001). Pearl mullet, which is an endemic species, migrates from Lake Van the main habitat to the rivers in shoals in April and July in order to breed,

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and returns to the Lake after spawning (Danulat and Selcuk 1992).

Different species and numbers of microorganisms play a crucial role in the spoilage of the caught fishes, and fish contain many pathogenic microorganisms that cause food poisoning. In the studies, it has been determined that different fish species are contaminated with pathogenic microorganisms (Davies et al. 2001; Farber 1991; Heinitz and Johnson 1998; Lennon et al. 1984; Norrung et al. 1999; Vaz-Velho et al. 2001).

L. monocytogenes of *Listeria* species has been accepted as one of the most important food pathogens recently (Farber and Peterkin 1991), as it causes the diseases called as listeriosis in human and animals (Jones and Seeliger 1992). Listeriosis is mostly seen in pregnant women, infants, alcohol and drug addicts, elderly people and the immunosuppressed individuals (Bahk and Marth 1990; Farber and Peterkin 1991; Pinner et al. 1992; Schuchat et al. 1992) and the general clinical appearance of the disease is similar to meningitis or septicemia (Seeliger and Jones 1986).

Listeria species are present in various ambits such as environment, soil, feces, sewage, water, plants, animal feeds and nutrients (Bahk and Marth 1990; Bortolussi et al. 1985; Brackett 1988; Colburn et al. 1990). Mainly consumption of infected foods leads to the disease upon infection of the agent to human beings in listeriosis cases (Bahk and Marth 1990; Brackett 1988; Pinner et al. 1992; Schuchat et al. 1992). In addition to the environmental contamination in the listeriosis cases occurring due to the nutrients, preservation of the viability of *L. monocytogenes* poses significant risks in terms of public health (El-Kest et al. 1991; Walker et al. 1990). As the source of epidemics caused by *L. monocytogenes*, cabbage salad (Schlech et al. 1983), pasteurized milk (Fleming et al. 1985) and Mexican-type soft cheeses (Linnan et al. 1988) are primarily held responsible.

Until a research study reported that epidemic prenatal listeriosis was due to consumption of fresh fish and shellfishes (Lennon et al. 1984) and a case of sporadic listeriosis was due to consumption of undercooked fish (Facinelli et al. 1989), it was thought that aquatic products

were not to be so effective in listeriosis cases. After it is specified that aquatic products have a role in the infection of *L. monocytogenes* to humans, many researchers have examined the existence, prevalence and the reproductive abilities of *Listeria* species in seawater, sediment, fish and other aquatic products in various parts of the world (Adesiyun 1993; Colburn et al. 1990; Davies et al. 2001; Farber 1991; Fuchs and Surendran 1989; Gohil et al. 1995; Manoj et al. 1991; Norrung et al. 1999; Weagant et al. 1988).

In the studies on various nutrients provided for consumption in Van, *Listeria* species were isolated at different rates (Elibol 2003; Isleyici et al. 2006; Sağun et al. 2001; Sancak et al. 2003). However, no study on the presence of *Listeria* spp. in the pearl mullet was found. In this study, the presence and prevalence of *Listeria* species according to the seasons were examined in pearl mullet, which is an indispensable element with the amount of protein in the nutrition of the people living in the region and creates an important commercial source in family subsistence.

MATERIALS and METHODS

In this study, the pearl mullet (*Chalcalburnus tarichi*, Pallas 1811) samples taken from the market a total of 160 times for 12 months were used as a material. The samples taken in sterile bottles under aseptic conditions were taken to the laboratory in a cold chain (+4°C) and the analyses were started as soon as possible (Gökalp et al. 1995). After obtaining dorsal muscles of minimum five fish and parts taken from the skin, they were chopped thinly and mixed well and samples were taken from this mixture for analyses. The method recommended by USDA/FSIS was used to determine the *Listeria* species in pearl mullet samples (Cook 1998). pH values of homogenized fish samples were determined by using a micro pH-meter (Hanna, pH 211, Germany).

RESULTS

Distribution of *Listeria* species by seasons is as shown in Table 1.

Table 1. Distribution of *Listeria* species by seasons

Season	n	<i>Listeria</i> spp.	<i>L. innocua</i>	<i>L. ivanovii</i>	<i>L. welshimeri</i>
Summer	40	1	1	-	-
Autumn	40	2	-	1	1
Winter	40	8	3	1	4
Spring	40	9	6	-	3
Total	160	20	10	2	8

DISCUSSION

Identification of *Listeria* species in pearl mullet at the rate of 12.50% may be associated by the transfer of the fish caught under non-hygienic conditions and the contamination of these microorganisms from the environment during launching.

As a matter of fact, the caught fishes are brought to the distribution centers by filling in the case and drums, distributed here mostly to hawkers, with wheelbarrow, tricycle, washbowl and drums offered for sale in non-hygienic environments and at ambient temperature.

Even though Embarek et al. (1997) and Pullela et al. (1998) reported that *Listeria* species could not be isolated in the fish samples examined, many researchers have stated that various *Listeria* species are isolated in the fish (Adesiyun 1993; Buchanan et al. 1989; Davies et al. 2001; Farber 1991; Gohil et al. 1995; Manoj et al. 1991; Nitcheva et al. 1990; Vaz-Velho et al. 2001).

In this study, the isolation rate of *Listeria* determined in pearl mullet samples was found to be higher than the isolation rates determined in the fish by some researchers (Gohil et al. 1995; Nitcheva et al. 1990). The isolation rates reported by Manoj et al. (1991) and Adesiyun (1993) are similar to the results specified in this study. Various *Listeria* species were reported to be isolated in 15 (26.79%) of 56 fish samples by Vaz-Velho et al. (2001), in 3 (30.00%) of 10 fresh sea foods by Fuchs and Surendran (1989), in 35 (61.00%) of 57 frozen sea foods by Weagant et al. (1988), in 5 (28.00%) of 18 fish and shellfish samples by Buchanan et al. (1989), and 10 (31.25%) of 32 fish samples by Farber (1991). The fact that the isolation rate of *Listeria* determined in pearl mullet samples was lower than the rates reported in various aquatic products may be due to the fact that the Lake Van in which this fish inhabits is not a suitable environment for the living of microorganisms. Indeed, Embarek (1994) reported that *Listeria* species can be found naturally in freshwater fish, but are not likely to be found in open seas or in fishes inhabiting in fresh salt waters. Differences between the studies may be associated with the sampling time and conditions, regional differences and the different levels of environmental contamination. Also, Farber and Peterkin (1991) reported that the number of samples examined with the methods used for isolation and identification may cause the findings to be different.

Obtaining different results in pearl mullet samples examined in summer, fall, winter, and spring seasons is compatible with statements by Karunasagar and Karunasagar (2000) reporting that various results were obtained in studies conducted at the same laboratory in different seasons and this could be associated different fish species and seasons. The low *Listeria* isolation in summer and autumn may be caused by the suppression of the production of *Listeria* species of the mesophilic microorganisms in the fish offered for sale under ambient temperature or by the aggravation of isolation. Indeed, while Guyer and Jemmi (1991) reported that different microorganism intensity in fish may play an important role in the development of *Listeria* species, Varabiouff (1990) stated that the microflora in the environment, may play an important role in the development of *Listeria* species.

Colburn et al. (1990) stated that *Listeria* species were determined in the fresh water (81%), salt water (33%) and sediment (30.4%) samples examined. The researchers stated that the different isolation rates in the samples examined may be caused by the competitive flora in the samples and the different salt ratios in the water where the fishes inhabit and the effect of urbanization and the surrounding animals. Especially, they stated that domestic livestock near the sites where fresh water samples were taken were observed and they may have contributed to the *Listeria* incidence identified in the samples. In this study, maximum *Listeria* isolation in the samples examined was detected in the spring season. The high rate of isolation in spring strengthens the possibility that fish migrating towards rivers to spawn in the spring season may be caught from rivers which are thought to be contaminated with the feces of surrounding animals and are a more suitable environment for the inhabitation of *Listeria*

species than Van Lake. High *Listeria* isolation rate in the samples examined in the winter season suggests that *Listeria* species may have been contaminated by the environmental sources at the stages after the fish were caught.

The fact that *L. innocua* (6.25%) was determined as dominant species in pearl mullet samples shows similarities opinions of the researchers reporting in different studies that *L. innocua* was found to be the dominant species in meat and seafood (Erol and Şireli 1999; Erol et al. 1999; Fuchs and Surendran 1989; Genigeorgis et al. 1989; Gohil et al. 1995; Nitcheva et al. 1990; Vaz-Velho et al. 2001; Weagant et al. 1988), with opinions of Şireli and Erol (1999) that *L. innocua* was more dominant in Turkey. Also, Petran and Swanson (1993) specified that *L. innocua* was produced more than *L. monocytogenes* in UVM and FB and accordingly, *L. innocua* became the predominant type within the colonies in the selective medium in which inoculation was done for isolation purposes. The fact that the enrichment broths used by the researchers are the same as the broths used in this study may have caused *L. innocua* to be isolated at a higher rate. Some researchers (Genigeorgis et al. 1989; Skovgaard and Morgen 1988) have reported that in meat products *L. innocua* is present 2-3 times more than *L. monocytogenes* and they have explained this by the shorter generation time of *L. innocua* compared to *L. monocytogenes*.

While the isolated species in the pearl mullet samples examined was mostly *L. innocua*, *L. monocytogenes* was not identified in any sample. This is compatible with the opinions of Curiale and Lewus (1994) who have reported that the generation period of *L. innocua* is shorter compared to *L. monocytogenes* and isolation of *L. monocytogenes* would be lower when there is *L. innocua* in the environment. However, the fact that *L. monocytogenes* was not isolated in any sample in this study is similar to the results of Fuchs and Surendran (1989) and Manoj et al. (1991), who reported that *L. monocytogenes* could not be isolated in the fish samples examined.

In a study (Boynukara et al. 1995) conducted in and around the Lake Van, it was reported that the total number of aerobic mesophilic microorganisms of the Lake Van was maximum 4.35×10^4 cfu/ml in the areas close to the settlement areas and maximum 1.96×10^3 cfu/ml in the areas distant from the settlement areas. Since there is no suitable environment for production of microorganisms due to alkaline and salt waters of the Lake Van and especially waters in the areas distant from settlement areas contain small amount of microorganisms, this made us think that fish were contaminated at stages until they were presented to consumer after they were caught. *Listeria* species, which are accepted to be the microorganisms that can be found almost everywhere in nature (Jones and Seeliger 1992) and whose source of aquatic organisms is estimated to be the environment (Karunasagar and Karunasagar 2000), may have contaminated the fish examined in this study by the environmental sources.

Andre and Genicot (1987) reported that *L. welshimeri* is particularly associated with environmental sources and rarely with the animal sources. Mostly the isolation of *L. welshimeri* (5.00%) after *L. innocua* (6.25%) in the pearl mullet samples strengthened the possibility that contamination may be associated with environmental sources and this result is similar to opinions of Buchanan

et al. (1989) reporting that *L. welshimeri* is a common contaminant in meat products and seafood.

The average pH values of 6.70 determined in the samples examined in this study was found between the pH values of 6.0-9.0 at which *Listeria* species can produce (Seeliger and Jones 1986). The average pH values reported in previous studies performed on fresh pearl mullet (Kılınççeker and Küçüköner 2003; Küçüköner et al. 2001) are slightly lower than the average pH value determined in this study and between the pH values that *Listeria* species can produce.

CONCLUSION

This study the presence of *Listeria* species in pearl mullet, submitted for consumption in Van, has been revealed for the first time, however, *L. monocytogenes*, a pathogenic species for humans, was not identified in any sample. The fact that *L. monocytogenes* could not be identified in any of the examined samples in this study can be evaluated as an indicator that pearl mullet does not constitute a public health risk for listeriosis. In order to determine the actual contamination level in the pearl mullet caught from the lake, the presence of *Listeria* species, especially *L. monocytogenes*, should also be investigated in other settlement areas where the fish is consumed. To avoid *L. monocytogenes* and other pathogen microorganisms from posing a public health risk, transport, storage, and marketing process of the caught fishes should be done under hygienic conditions and cold.

CONFLICT of INTEREST

The authors declare that they have no conflict of interest.

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