

Incidence of *Listeria* Species in Farmed Tropical Fish in Khuzestan, Iran

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Abstract: The incidence of *Listeria* spp. was determined in freshly-harvested, farmed fish (grass carp and common carp) in Khozestan, South-West Iran. One hundred fish samples were purchased at the fish markets, in the warm and cold seasons. Fish samples' intestines and gills were examined for *Listeria* spp. 18 samples (18%) were positive for *Listeria* spp. isolation. In the warm season, 2 samples of Grass carp fishes were positive for *Listeria monocytogenes* and 3 and 4 samples of both grass carp and common carp fishes were positive for *L. grayi* and *L. innocua* respectively. In the cold season, again 2 samples of grass carp fishes were positive for *L. monocytogenes* and 7 samples for *L. innocua*. no common carp samples were contaminated with *Listeria* spp. This frequency of contamination of fresh farmed fish with *L. monocytogenes* in Khuzestan is a concern and the handling and consumption of these fish, either raw or undercooked, may pose a health risk.

Key words: Incidence • *Listeria* • Tropical • Fish • Iran

INTRODUCTION

Listeria spp. are widespread in the environment and commonly found in soil, sewage, dust and water. One particular species, *Listeria monocytogenes*, has been recognized as a food-borne pathogen since 1981. It is known as the causal agent of listeriosis, which is characterized by fever, chills, convulsions, headache, diarrhea and vomiting which are commonly flu-like symptoms [1]. The bacterium can grow in many foods, even at the refrigerator temperature. The organism has been isolated from freshwater and seawater fish and from frozen and processed sea food [2-7].

A number of food-borne outbreaks and sporadic cases of listeriosis has been reported mostly in North America and Europe [9]. Persons most susceptible to the disease are immunocompromised hosts such as pregnant women and the elderly.

L. monocytogenes and other *Listeria* spp. have been isolated from fishery products on a regular basis in tropical and temperate regions since 1980s. *L. monocytogenes* is considered as a psychrotrophic pathogen and may thus be less common in tropical water. Ben Embarek [10] reviewed the incidence of *Listeria* in seafood worldwide and found that the prevalence of *L. monocytogenes* varies from 4 to 12% in temperate areas.

Some reports [11-13] have suggested the absence of *L. monocytogenes* in tropical fishes, but Ben Embarek [10] emphasized that more research is needed regarding the incidence of *L. monocytogenes* in tropical seafood. Adesiyun [14] noted a 2% incidence of *L. monocytogenes* in seafood samples from Trinidad. In India, the prevalence of *L. monocytogenes* in tropical fresh shellfish and finfish ranges from 12.1 to 17.2% [15]. In Latin America, the prevalence of *L. monocytogenes* in fish and fish products varies from 0 to 50% [16] and, in India, 9% of fresh/raw seafood samples are contaminated with *L. monocytogenes* [17]. Thus, it may be concluded that *Listeria* can be an important problem even for fisheries in tropical countries.

There are limited data regarding the prevalence of *L. monocytogenes* in seafood consumed in Iran and no information exists on the incidence of *Listeria* spp. in fresh farmed fish in Khuzestan province. *L. monocytogenes* was detected in 6.6 to 10% of common carp and silver carp samples and in 11.6 to 12.5% of farmed rainbow trout, in Tehran and Gilan province in the temperate north of Iran [18]. Other studies in Iran have reported low prevalence of *L. monocytogenes* in frozen and fresh fish samples ranging from 0 to 2.6% [19-20] but the incidence of the bacterium in traditional smoked salmon in Gilan is high, at 34% [21].

The purpose of the present study was to generate information on the incidence of *Listeria* spp. in the freshwater fish in Khuzestan province in South-West Iran, which has a tropical climate for at least 8 months of the year and is a major centre for the culture and export of freshwater fish.

MATERIALS AND METHODS

Samples: During November 2008 to October 2009, one hundred fish samples, 50 common carp (*Cyprinus carpio*) and 50 grass carp (*Ctenopharyngodon idella*) were purchased at the local fish markets of Ahvaz City, Khuzestan, Iran. Fish samples were collected from the local fish farms and transported on ice to the fishmongers within 24 h of capture. All samples were placed in sterile bags, on ice, for transport to the laboratory and processed within 2 h of collection.

Isolation and Identification of *Listeria* spp: Samples were analyzed for *Listeria* spp. using the enrichment, selection and isolation protocol recommended by the US Food and Drug Administration [22]. For each sample, 5g of intestine and 2-3 gills were removed aseptically and placed separately in 20 ml of *Listeria* Enrichment Broth (LEB, Merck, Germany) containing potassium thiocyanate (37.5 g/L, Merck). In a class III safety cabinet, this mixture was homogenized and then incubated at 35° C. On the same day and after 24h and 48h, 100 iL samples from the enrichment culture were plated onto *Listeria* selective agar (Oxford formulation, Himedia, India) and incubated at 37° C for 48h. The plates were examined for typical *Listeria* colonies (translucent greenish yellow colonies) and at least 3 suspected colonies were sub-cultured on Brain Heart Infusion Agar (BHIA, Hispanlab) and incubated at 37° C for 48h. All the isolated colonies were characterized by Gram staining, motility at 25° C and 37° C, catalase and

oxidase tests and acid production from xylose and mannitol. For further confirmation; other biochemical reactions, α -hemolytic activity on 5% sheep blood agar (Merck) and CAMP test were performed according to Bergey's Manual of Systematic Bacteriology [23].

Serotyping of the Confirmed *Listeria* Isolates:

Serotyping by slide agglutination was determined using Bacto-*Listeria*-O antisera types 1 and 4 (Difco, USA). Complete serotyping of confirmed isolates was performed in the Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, using "in house" production reagents as described elsewhere [24].

RESULTS AND DISCUSION

Our study showed that 18 out of the 100 (18%) tested samples from grass carp and common carp were positive for *Listeria* spp (Table 1). In the cold season (From November to March), 2 isolates from grass carp fishes were *L. monocytogenes* and 7 isolates were identified as *L. innocua*. In the warm season (From April to October), 2 samples of grass carp fishes were *L. monocytogenes* and 3 and 4 samples of both grass carp and common carp fishes were positive for *L. grayi* and *L. innocua* respectively. *L. innocua* was detected in 11% of samples and was the predominant isolate among the *Listeria* spp. This agrees with the findings of Jalali and Abedi [20], Parihar *et al.* [17] and Dhanashree *et al.* [25]. Since both *L. monocytogenes* and *L. innocua* share the ecological niches, the isolation of both bacteria is not surprising and isolation of *L. innocua* in fishery products is considered as an indicator of possible contamination with *L. monocytogenes* [26]. *L. grayi* was found in 3 samples (3%) only in the warm season and there are no previous reports of isolation of this strain in Iranian seafood. Failure to isolate *L. grayi* in the cold season needs more

Table 1: Incidence of *Listeria* species in fish samples

		Number and (%) of samples positive for		
Fish	Number of samples	<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. grayi</i>
Cold session				
Grass carp	25	2(8) ^a	7(28)	-
Common carp	25	-	-	-
Warm session				
Grass carp	25	2(8) ^b	2(8)	1(4)
Common carp	25	-	2(8)	2(8)
Total	100	4(4)	11(11)	3(3)

a Both strains were serotype 1/2 a and were isolated from gut and gills

b One strain was serotype 1/2 a and one was serotype 4b and were isolated from gills

investigation. The location of *Listeria* spp. and *L. monocytogenes* in different parts of fish differed with statistical significance in samples. Up to 75% of the *L. monocytogenes* and 83.3% of *Listeria* spp. positive samples were gill samples. Also, It can be stated that only grass carp fishes were contaminated by *L. monocytogenes*.

The serotypes of fish-associated *L. monocytogenes* have been reported by few authors. Adesiyun [14] found that all the strains isolated from seafood were serotype 4b whereas the tropical isolates of *L. monocytogenes* belonged to serotypes 1 and 4 [27]. Basti *et al.* [19] noted that *L. monocytogenes* isolated from fresh, smoked and salted Iranian fish belong to either serovar 1/2a or 1/2b. Our results showed that the isolates of *L. monocytogenes* from farmed tropical fish belonged to serotypes 1/2a and 4b. The serotypes of *L. monocytogenes* more commonly associated with sporadic cases and outbreaks of listeriosis are 4b, 1/2a and 1/2 b, accounting for more than 90% of all cases reported [28]. All our isolates of *L. monocytogenes* were 1/2a and 4b; this underscores of food items such as farmed tropical fish to cause listeriosis. The increased number of listeriosis cases over the last few years and the fact that the majority of strains were identified as virulent in our study, indicate that the present HACCP-based strategy should be readjusted to take this in to consideration.

Khuzestan province is one of the major regions in Iran for culture and export of freshwater fish. Most of the fish farms are situated near Karone, the biggest fresh water river in Iran and use the river as water intake. Isolation of *Listeria* spp. from the gut and gills of fishes indicates that the fishes are contaminated via the river or by the animal feces used for fertilizing the farm ponds. The river also could be contaminated with *L. monocytogenes* from farm sewage or other sources.

In conclusion, isolation of *L. monocytogenes* in tropical fish in this study indicates that there is a risk of acquiring listeriosis through this product in Iran. Although the bacterium will be killed by sufficient cooking, it may pose a health risk in the kitchen or restaurants by cross-contamination of cooked food or other kinds of ready-to-eat food. Using a risk analysis approach, research should be carried out to assess the prevalence of *L. monocytogenes* in other kind of fresh water and seawater fishes, also in frozen, smoked and lightly preserved fish product in Khozestan province.

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