

JOURNAL OF HALAL QUALITY AND CERTIFICATION

Microbiological Correctness of Fish with Reference to *Listeria Monocytogenes* in The Context of Importance to Halal Production

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Scientific paper

ARTICLE INFO

ABSTRACT

Keywords:

halal quality, *Listeria monocytogenes*, rainbow trout

The finding of microorganisms, especially pathogens, in food determines the correctness of food and the possibility of using it in human nutrition. According to the current legislation of Bosnia and Herzegovina, food of animal origin is considered microbiologically defective if they contain an impermissible number of pathogens, among which *Listeria monocytogenes* occupies a special place in the context of public health. Listeriosis in humans is characterized by gastrointestinal disorders, the occurrence of meningitis and miscarriages in pregnant women. The aim of the research is to examine the prevalence and seasonality of *Listeria monocytogenes* in rainbow trout (*Oncorhynchus mykiss*) in order to confirm the halal quality of the product. In the production and processing of fish, critical control points were monitored and the presence of *Listeria monocytogenes* was determined. A total of 320 samples of rainbow trout sampled at different stages of production and processing were analyzed, using microbiological methods according to the international standard BAS EN ISO 11290-1/A1:2005. The total number of rainbow trout samples where *Listeria monocytogenes* was detected was 3,12%. Prevention includes checking the status of water and food that fish consume, as well as work and production hygiene. It is necessary to avoid cross-contamination, one of the main ways of contamination in production. Fish contaminated with pathogens, such as *Listeria monocytogenes*, is not allowed for human consumption due to its harmfulness to human health, and therefore such fish does not meet the criteria of a halal product.

1. Introduction

Listeria monocytogenes is a ubiquitous bacterium in nature, so the mode of transmission is quite broad. The relationship between this bacterium, the environment, animals, humans and food is not entirely clear, but the fact that it

is transmitted through food and can be dangerous to human and animal health, positions it as a priority in the field of food safety (Aala et al. 2023; Samanta & Choudhary 2019). Protection against pathogens is based on prophylaxis (Ji et al. 2023). This is also stated in the reports of the Food and Drug Administration (FDA), the Food Safety and Inspection Service

(FSIS) and the American Department of Agriculture (USDA) with the aim of introducing a zero tolerance level on the presence of *L. monocytogenes* in ready meals (Antoci et al. 2021; Buchanan et al. 2017).

In a public health context, *Listeria monocytogenes* causes the disease listeriosis (Schoder et al. 2023). Clinically, we differentiate between invasive and non-invasive forms of listeriosis. Invasive listeriosis is characterized by a more severe clinical picture, mostly noticeable in children, pregnant women, the elderly and people with weakened immunity due to the presence of some chronic disease (Jordan et al. 2015). Invasive listeriosis is characterized by a high degree of mortality, while non-invasive listeriosis is dominated by febrile listeriosis gastroenteritis (Rivera-Izquierdo et al. 2023). This gastroenteritis occurs as part of listeriosis epidemics with symptoms in the form of diarrhea, fever, headache, muscle pain, etc., which occur after a short incubation period (Islam et al. 2023). In order to prevent contamination with *Listeria monocytogenes* in the production and processing of fish, attention should be paid to the microbiological status of the water and food consumed by the fish, as well as to the hygiene of work and production. It is necessary to pay attention to cross-contamination as one of the basic ways of contamination of final products in production chains (Zakrzewski et al. 2023).

The term halal in Islam represents everything that is permitted, and this also applies to food products that Muslims can consume. The opposite of the term halal is the term haram, which means anything that is forbidden in Islam. When we say that something is forbidden (haram), we can also say that it is not healthy for the human body, because according to Islamic laws, non-halal food is considered to transmit diseases. Halal standards dictate that food must meet all regulations on health, hygiene and sanitary correctness, that it does not contain ingredients that Muslims are forbidden to consume, and that no Sharia-prohibited raw materials and means were used in production

(Nazaruddin et al. 2023). Halal food must not contain pathogenic microorganisms (Maqsood & Ayyub 2023). In order for the fish to meet the criteria of a halal product, it must also be microbiologically correct. Therefore, the aim of this research is to examine the presence of *Listeria monocytogenes* in rainbow trout (*Oncorhynchus mykiss*) in order to confirm the halal quality of the product.

2. Material and methods

2.1. Research area

Rainbow trout (*Oncorhynchus mykiss*) samples were collected in two facilities, from a fish farm and a fish processing plant. The first facility is implemented with Halal/HACCP standards (facility A), while the second facility is not certified/registered in the veterinary-sanitary control system and does not comply with standards (facility B). Sampling was done in two seasons: spring-summer and autumn-winter. Isolation and identification of *Listeria monocytogenes* was carried out in the Laboratory for Microbiological Testing of Foodstuffs, Animal Feed and Items of General Use at the Veterinary Faculty of the University of Sarajevo. Sample analysis was done in accordance with the cold chain according to BAS EN ISO 11290-1/A1:2005.

2.2. Materials, samples of rainbow trout (*Oncorhynchus mykiss*)

The research included 320 samples of edible rainbow trout (*Oncorhynchus mykiss*). Seasonal sampling was carried out in such a way that in the spring-summer season 80 samples of edible rainbow trout were sampled in facilities A and B. The same sampling procedure of 80 samples of edible rainbow trout was repeated in both facilities in two seasons: spring-summer and autumn-winter (Table 1). The samples were cut with a sterile knife in the amount of 25.0 ± 0.5 g. and stored in vacuum plastic bags at a temperature of 4 ± 2 °C.

Table 1. Number of rainbow trout samples according to facility type and sampling season

Number of samples/season	Number of samples according to object type and sampling season	
	Facility A (Halal/HACCP)	Facility B (without Halal/HACCP)
Number of samples/spring-summer	(n=80)	(n=80)
Number of samples/autumn-winter	(n=80)	(n=80)
Total	(n=160)	(n=160)

2.3. Research methods

For the preparation of samples of edible rainbow trout (*Oncorhynchus mykiss*) for microbiological analysis, the substrates prescribed by the method BAS EN ISO 6887-1:2005 (ISO 6887-1 2005) and 6887-3:2005 (ISO 6887-3 2005) were used. All samples were transferred to a sterile Stomacher bag to which 225 mL of physiological peptone solution was added. All samples were serially diluted ten times. *Listeria* selective agar plates according to Ottaviana and Agosta (ALOA agar) (ISO) (VWR Chemicals BDH, Leuven, Belgium) were used for the cultivation of samples (100 µL). Incubation was done for 48-72 h, at 37°C.

For the isolation and identification of *Listeria monocytogenes*, the substrates prescribed by the method BAS EN ISO 11290-1/A1:2005 – Horizontal method for the detection and counting of *Listeria monocytogenes* – Part 1 (ISO 11290-1 2005) were used. The following substrates and diagnostics were used to perform this method: Half Fraser broth with supplements (Conda lab), Fraser broth (Conda lab), *Listeria* agar according to Ottaviana and Agosti (ALOA agar) (Conda lab), PALCAM agar (Conda lab), Tryptone soy yeast extract agar (TSYEA) (Conda lab), Tryptone soy yeast extract broth (TSYEB) (Conda lab), Sheep blood agar (Conda lab), Carbohydrate utilization broth (rhamnose and xylose) (Conda lab), Motility agar (Motility agar) (Conda lab), Christie, Atkins, Munch-Petersen) test strains, Phosphate-buffered saline (PBS), 0.85% NaCl solution. The following reference strains were used for isolation and identification of *L. monocytogenes* and quality control of microbiological media: *Listeria*

monocytogenes 4b (WDCM 00021), *Escherichia coli* (WDCM 00012), *Staphylococcus aureus* (NCTC 1803), *Rhodococcus equi* (NCTC 1621), *L. innocua* (ATCC 33090) and *L. ivanovii* (ATCC 19119).

3. Results

The results of research on the presence of *Listeria monocytogenes* in samples of edible rainbow trout (*Oncorhynchus mykiss*) in a facility with implemented Halal/HACCP standards (facility A) and in a facility that is not certified/registered in the veterinary-sanitary control system and does not comply with standards (facility B) are presented in tables 2 and 3 and graphs 1-4. In the facility with implemented HACCP and Halal standards, positive samples of rainbow trout for *Listeria monocytogenes* were recorded only in the spring-summer season at the slaughter and evisceration line (2/20, 10%). In a facility that does not comply with Halal standards, the highest number of positive samples for *L. monocytogenes* was recorded in both seasons on the slaughter and evisceration lines. The largest number of positive samples was recorded in the spring-summer (3/20, 15%), and slightly less in the autumn-winter (1/20, 5%). In the spring-summer season, positive samples were also recorded in a container with ice (1/15, 6.66%), frozen fish (1/15, 6.66%), while in the autumn-winter season, positive samples were from fish farms (1 /15, 6.66%) as well as fish from cassettes ready for transport (1/15, 6.66%).

Table 2. Representation and seasonality of *Listeria monocytogenes* in samples of edible rainbow trout in a facility with implemented Halal/HACCP standards (facility A)

Location/number of samples	Number of positive samples (%)	
	Spring-summer	Autumn-winter
Fish farms (n=15)	0 (0.00)	0 (0.00)
Containers with ice (n=15)	0 (0.00)	0 (0.00)
Slaughter and evisceration line (n=20)	2 (10.00)	0 (0.00)
Fish from the cache ready for transport (n=15)	0 (0.00)	0 (0.00)
Frozen fish (n=15)	0 (0.00)	0 (0.00)
Total (n=80)	2 (2.5)	0 (0.00)

Of the total number of rainbow trout samples in establishments with implemented Halal/HACCP standards (facility A), positive samples for the presence of *Listeria monocytogenes* were recorded only in the spring-summer season (2/80, 2.5%). In the case of samples taken in a

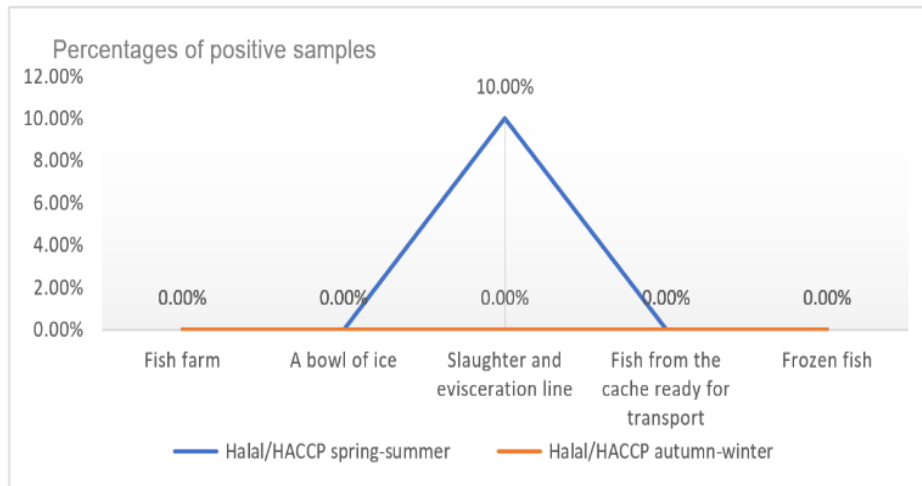
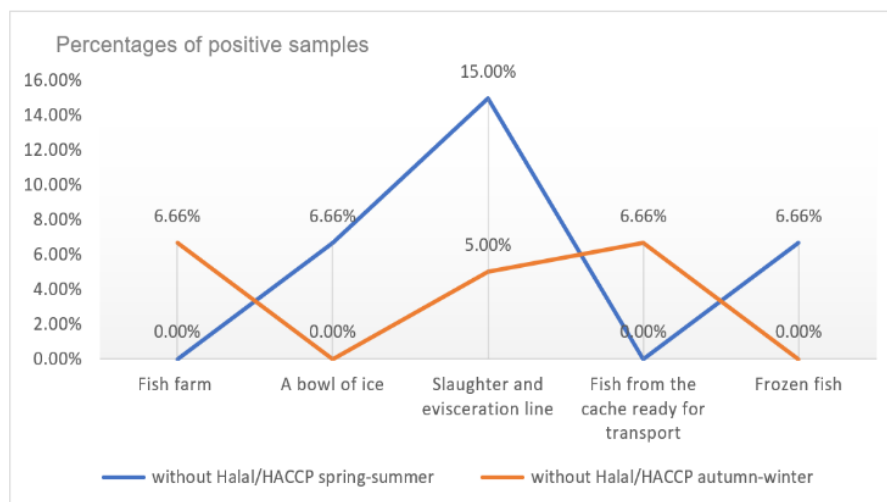
facility that does not comply with Halal/HACCP standards (facility B), the total number of positive samples was recorded in the spring-summer season (5/80, 6.25%) and almost half as much in the fall-winter season (3/ 80, 3.75%).

Table 3. Representation and seasonality of *Listeria monocytogenes* in samples of edible rainbow trout in a facility without implemented Halal/HACCP standards (facility B)

Location/number of samples	Number of positive samples (%)	
	Spring-summer	Autumn-winter
Fish farms (n=15)	0 (0.00)	1 (6.66)
Containers with ice (n=15)	1 (6.66)	0 (0.00)
Slaughter and evisceration line (n=20)	3 (15.00)	1 (5.00)
Fish from the cache ready for transport (n=15)	0 (0.00)	0 (0.00)
Frozen fish (n=15)	1 (6.66)	0 (0.00)
Total (n=80)	5 (6.25)	3 (3.75)

Of the total analyzed rainbow trout samples collected in facilities with and without Halal standards (Facilities A, B), the total number of

samples with *Listeria monocytogenes* is (10/320, 3.12%) (Graph 1-4).

Graph 1. Comparative presentation of the results of the presence and seasonality of *Listeria monocytogenes* in rainbow trout samples in a facility with implemented Halal/HACCP (facility A)Graph 2. Comparative presentation of the results of the presence and seasonality of *Listeria monocytogenes* in rainbow trout samples in a facility without implemented Halal/HACCP (facility B)

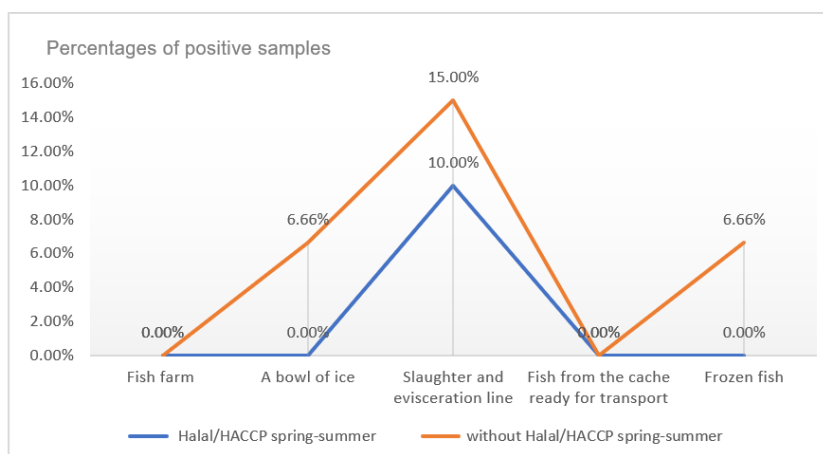


Chart 3. Comparative presentation of the results of positive samples for *Listeria monocytogenes* in facilities with and without implemented Halal/HACCP in the spring-summer season (facility A, B)

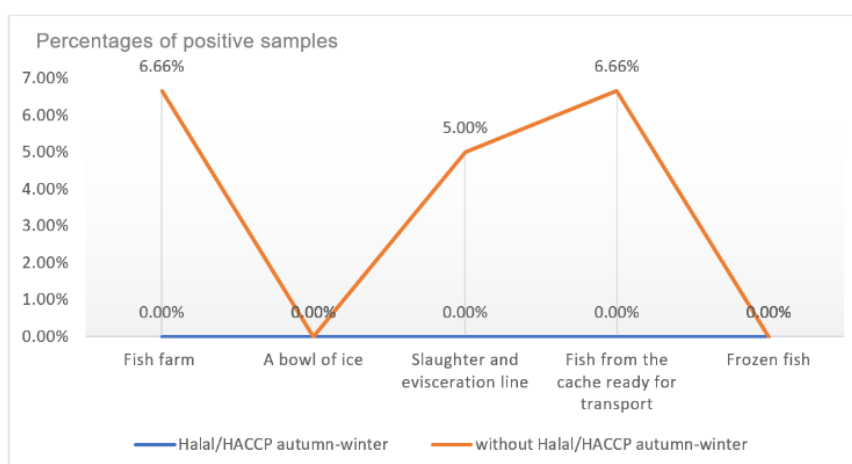


Chart 4. Comparative presentation of the results of positive samples for *Listeria monocytogenes* in facilities with and without implemented Halal/HACCP in the autumn-winter season (facility A, B)

4. Discussion

The aim of the research is to examine the prevalence and seasonality of *Listeria monocytogenes* in rainbow trout (*Oncorhynchus mykiss*) in order to confirm the halal quality of the product. It was observed that in the spring-summer season in facilities with implemented Halal/HACCP standards (facility A), a smaller percentage of positive samples for *Listeria monocytogenes* was present, while a complete absence of positive samples was recorded in the fall-winter season. The amount of *Listeria monocytogenes* in samples of edible rainbow trout increased in the spring-summer season, which confirms the seasonality of this pathogenic bacterium, which is especially visible in positive samples collected in facilities that are not certified/registered in veterinary-

sanitary control and do not comply with Halal/HACCP standards (Facility B), which indicates that halal products are of better quality in terms of sanitary quality. In contrast to the samples of edible rainbow trout that were collected in facilities with implemented Halal/HACCP standards, in facilities without implemented Halal/HACCP standards, positive samples are present in both seasons. All of the above indicates that halal products have a better microbiological status. Good microbiological quality is achieved with the help of Halal standards and with the help of the HACCP concept that includes *Good Manufacturing Practice* (GMP) and *Good Hygienic Practice* (GHP). A similar study of the prevalence and seasonality of *Listeria monocytogenes* in edible rainbow trout (*Oncorhynchus mykiss*) was conducted in Croatia, where Rožman et al. (2016) state that in facilities with implemented

Halal and HACCP standards, they obtained negative results in the autumn-winter season.

Listeria monocytogenes was detected in samples of edible rainbow trout in a facility without implemented Halal/HACCP standards in the spring-summer season, when 80 samples were analyzed. One positive sample was from the ice bin (1/15, 6.66%), followed by frozen fish (1/15, 6.66%), while three positive samples were from the slaughter and evisceration line (3/20, 15%). The total percentage of positive samples for *Listeria monocytogenes* from that sampling period was (6.25%) (Table 3). The results of this study are in the range of values reported in similar studies, so Rahimi et al. (2011) state that *Listeria* spp. isolated in (7.5%) samples of raw and frozen fish, of which *Listeria monocytogenes* was present in (1.9%) of the tested samples, while Lakičević et al. (2015) reported that (12.4%) positive samples for *L. monocytogenes* were present in samples of fresh fish, cold and hot smoked trout. Some authors state that *Listeria* spp. can be present in smoked products up to (39.4%), while recent research records the percentage of positive samples in smoked products in the range of (0-12%) (Rotariu et al. 2014). Similar results were found in fresh and frozen fish samples by other authors in Japan, Ryu et al. (1992), Sweden, Parihar et al. (2008) and India, Dhanashree et al. (2003). Research indicates that hygienic and sanitary standards in production processes reduce the contamination of final products. Contamination reduction is also achieved by proper fish processing, thermal processing and freezing (Rotariu et al. 2014; Ziarati et al. 2022). It should be emphasized that the ice used in cold chains must be free of *L. monocytogenes* (Wang et al. 2023).

Listeria monocytogenes was detected in the autumn-winter season in rainbow trout samples from a facility without implemented Halal/HACCP, where out of a total of 80 samples taken, it was positive (3.75%). Out of the total number of samples from farms with rainbow trout, one positive sample was detected for *Listeria monocytogenes* (1/15 6.66%), at the slaughter and evisceration line (1/20.5%) and fish from caches ready for transport (1/15, 6.66%) (Table 3). The results shown in Tables 2, 3 and 4 agree with the results of other authors, who also found a different number of positive fish samples for the presence of *Listeria monocytogenes* (17.2%) Jeyasekaran et al. (1996), as well as Kuzmanović et al. (2011) who found positive samples of fresh fish, fish products and seafood for the presence of *L.*

monocytogenes (1.92%).

By comparing the detection results of *Listeria monocytogenes* in samples of rainbow trout from a facility with Halal and HACCP standards in the spring-summer and autumn-winter period, it is evident that in the spring-summer period the percentage of positive results is (10%), while in the same facility no *L. monocytogenes* was detected in the autumn-winter period (Graph 1). Graph 2 shows a comparison of the detection results of *L. monocytogenes* in rainbow trout samples from a facility without Halal and HACCP standards in the spring-summer and autumn-winter periods. The total percentage of positive samples from the spring-summer period (28.32%) was higher compared to the percentage of positive samples from the same facility from the autumn-winter period (18.32%), which may suggest that increased surveillance is needed in the summer period. By comparing the results of detection of *Listeria monocytogenes* in rainbow trout samples from a facility with implemented Halal/HACCP standards in the spring-summer and fall-winter season, it is evident that in the spring-summer season the percentage of positive results (10%), while in the same facility no *L. monocytogenes* in the autumn-winter period (Graph 1). Graph 2 shows a comparison of the detection results of *L. monocytogenes* in rainbow trout samples from a facility without implemented Halal/HACCP standards in the spring-summer and fall-winter seasons (Graph 3). The largest number of positive samples for *L. monocytogenes* was detected at the slaughter and evisceration line, because according to Papadopoulos et al. (2010), at that sampling point contact with fish by processors increased. In fish processing facilities, subsequent contamination occurs as a consequence of evisceration, gill removal, shell cleaning, filleting, which agrees with our results. Elischerova et al. (1979) report the results of research from Slovakia, where the largest number of positive samples for *L. monocytogenes* were on the hands of workers during the smoking process (14.5%) and the meat cutting process (8.9%). Graph 4 compares the results of rainbow trout samples from establishments with and without implemented Halal/HACCP standards in the fall-winter season. Although positive results are evident in the facility without implemented standards at three sampling sites (Slaughter and evisceration line, containers with ice, frozen fish and fish from cache ready for transport) (18.32%), compared to the facility with implemented Halal/HACCP standards where *L.*

monocytogenes was not detected, but the difference was not statistically significant, but was the result of non-systemic variable factors. Sanitation procedures can reduce or destroy a significant amount of *Listeria monocytogenes* from the production line as well as from equipment, but recontamination can occur shortly after starting a new production cycle. This is linked to the formation of a biofilm that increases resistance and allows the bacterial cell to survive in unfavorable microenvironmental conditions (Mazaheri et al. 2023). Given that *Listeria monocytogenes* quickly adapts to environmental conditions, contamination during production, distribution and retail cannot be ruled out, which poses a challenge in controlling *L. monocytogenes* despite the application of all prescribed sanitary measures (Bolívar et al. 2023). Production processes and facilities must be under control and hygienic-sanitary processing, in order to reduce the possibility of product recontamination to the lowest level during production (Nguyen Trang et al. 2023).

5. Conclusion

The research confirmed the presence of *Listeria monocytogenes* in samples of rainbow trout (*Oncorhynchus mykiss*) from ponds and processing facilities, which indicates the possibility of contamination of the final products. Positive samples at the slaughter and evisceration line indicate the obligation of constant supervision and implementation of hygienic and sanitary measures in accordance with Halal/HACCP standards. Fish contaminated with pathogens, such as *Listeria monocytogenes*, is not allowed for human consumption due to its harmfulness to human health, and therefore such fish does not meet the criteria of a halal product.

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Mikrobiološka ispravnost riba sa osvrtom na *listeria monocytogenes* u kontekstu značaja na halal proizvodnju

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Naučni rad

PODACI O RADU

SAŽETAK

Ključne riječi:

halal kvaliteta,
kalifornijska pastrmka,
Listeria monocytogenes.

Nalaz mikroorganizama, patogena u hrani određuje ispravnost hrane i mogućnost korištenja u ljudskoj ishrani. Prema važećem zakonodavstvu Bosne i Hercegovine, namirnice animalnog porijekla smatraju se mikrobiološki neispravnim ako sadrže nedozvoljeni broj patogena, među kojima u kontekstu javnog zdravstva posebno mjesto zauzima *Listeria monocytogenes*. Listeriozu kod ljudi karakterišu gastrointestinalni poremećaji, pojava meningitisa i pobačaji kod trudnica. Cilj istraživanja je ispitati zastupljenost i sezonalnost *Listeria monocytogenes* u kalifornijskoj pastrmci (*Oncorhynchus mykiss*) u svrhu potvrde halal kvaliteta proizvoda. U proizvodnji i preradi ribe praćene su kritične kontrolne tačke i utvrđeno je prisustvo *Listeria monocytogenes*. Analizirano je 320 uzoraka kalifornijske pastrmke uzorkovane u različitim fazama proizvodnje i prerade, uz korištenje mikrobioloških metoda prema standardu BAS EN ISO 11290-1/A1:2005. Ukupan broj uzoraka kalifornijske pastrmke kod kojih je otkrivena *Listeria monocytogenes* iznosi 3,12%. Prevencija uključuje provjeru stanja vode i hrane koju ribe konzumiraju, kao i higijenu rada i proizvodnje. Potrebno je izbjeći unakrsnu kontaminaciju, jedan od osnovnih načina kontaminacije u proizvodnji. Riba koja sadrži patogene, među kojima je i *Listeria monocytogenes*, nije dozvoljena za ljudsku ishranu zbog štetnosti po zdravlje ljudi, te stoga takva riba ne ispunjava kriterije halal proizvoda.
