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Fish Product–Borne Histamine Intoxication Outbreak and Survey of Imported Fish and Fish Products in Serbia

JELENA PETROVIC,* JELENA BABIĆ, SANDRA JAKSIC, BRANKICA KARTALOVIC, DRAGANA LJUBOJEVIC, AND MIROSLAV CIRKOVIC

Scientific Veterinary Institute "Novi Sad," Rumenacki put 20, 21000 Novi Sad, Republic of Serbia

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ABSTRACT

Histamine levels in fish and fish products are regarded as a primary criterion for food safety, considering the effects of histamine on human health. The aim of this research was to describe a case of massive histamine intoxication in Serbian children and to provide relevant information regarding the presence of histamine in imported marine fish and fish products available in the Serbian market. In January 2014, an incident of foodborne illness occurred in 28 children (aged 2 to 5 years) who consumed canned sardines in a kindergarten in Vojvodina province, northern Serbia. The diagnosis was established based on anamnestic data, epidemiological data, and clinical symptoms and confirmed by positive histamine finding in the incriminated sardines. Substantially high histamine levels (>300 mg/kg) were detected in seven of the nine examined units of these canned sardines. In addition, during an official veterinary border control from January 2013 to January 2015, 273 lots in total, including 2,457 fish and fishery product units, were analyzed using enzyme-linked immunosorbent assay. Even though only nine (3.29%) of all examined lots were declared as unsafe for human consumption, the presented case of histamine intoxication strongly suggests the importance of border inspection and comprehensive control of each imported lot.

Histamine [1*H*-imidazole-4-ethanamine] content in fish and fish products is a criterion for food safety, owing to the effects of histamine on human health. In addition, histamine is an important indicator of earlier decomposition of fish and process hygiene level. Histamine is a low-molecular-weight organic nitrogenous base, a biogenic amine mainly formed by decarboxylation of the amino acid histidine *(7, 15)*.

Histamine fish poisoning, also known as scombroid fish poisoning, pseudoallergic fish poisoning, histamine overdose, or mahi-mahi flush *(1),* represents a major histamine intoxication with fish and fishery products, implicating mainly the fish species with high histidine levels from the families Scombridae, Clupeidae, Engraulidae, Pomatomidae, and Scombresocidae *(11, 27)*. The most common fish in these families are tuna, mackerel, herring, and sardine *(16, 35)*. Because of the popularity and consumption of large quantities of fish from these families globally, histamine fish poisoning is identified as one of the most frequent fish poisonings in Europe and worldwide *(8)*.

Major factors contributing to a toxic reaction in consumers include the amount of dietary histamine ingested, the individual sensitivity of the consumer, and the detoxification activity of the body *(34)*. Most common manifestations of histamine intoxication involve changes in the cardiovascular system, predominantly because of the dilation of peripheral blood vessels, leading to urticaria, hypotension, flushing, and headache. Histamine intoxication occurs shortly after consuming (within a few minutes to 2 h) the food product and is characterized by a headache, nasal secretion, bronchospasm, hypotension, tachycardia, urticaria, edema, pruritus, and diarrhea *(10, 12, 17)*. Persons who got sick after consuming spoiled fish manifested much more severe symptoms compared to volunteers, who were administered the same oral doses of pure histamine. Thus, the presence of certain histamine potentiators in fish is assumed, and some other biological amines such as putrescine and cadaverine *(35),* tyramine, spermidine, and spermine *(17)* have been identified. According to reports from the Rapid Alert System for Food and Feed, more than 100 cases of histamine intoxication outbreaks in the European Union were documented from 2005 to 2010 *(7)*.

Freshly caught fish generally contain histamine at low physiological levels. The increase in histamine concentration occurs during processing and storage of the products *(6)*. Factors influencing histamine formation include the presence of histidine, appropriate pH, water activity, the presence of particular microorganisms, and a suitable ambient temperature *(16, 35)*. The formation of histamines in fish tissues is attributed to a range of microorganisms, such as *Hafnia alvei, Morganella morganii, Morganella psychrotolerans, Photobacterium phosphoreum,* and *Klebsiella pneumoniae (5, 14, 16)*. Mesophillic organisms are mostly considered responsible for histamine production at an optimum temperature of 20 to 37°C, producing more than 500 mg/kg after 48 h *(14)*; however, psychrophilic bacteria

* Author for correspondence. Tel: (381) 21 4895370; Fax: (381) 21 518544; E-mail: jelena@niv.ns.ac.rs.

(*Photobacterium iliopiscarium*) have been found to produce histamine at levels higher than 500 mg/kg after culture at 5°C for 3 days, 10°C for 36 h, and 20°C for 18 h *(25)*. The influence of pH on histamine production is twofold and quite intricate. Although low pH inhibits the growth of microorganisms, a low pH environment also stimulates decarboxylation and histamine formation. These opposing factors interfere with each other, and the final effect strongly depends on their balance *(7)*. Salt (sodium chloride) content affects histamine production in accordance with its inhibitory effects toward microbial growth of relevant bacteria, except for the halotolerant organisms, whereas decarboxylation of histidine seems to be inactivated or destroyed in the presence of oxygen *(13)*.

The implementation of the prerequisite programs and hazard analysis (and) critical control point concept into the fish processing industry are essential steps to reduce the risk of histamine occurrence. The prevention of contamination with aminogenic microorganisms is important because high histamine levels were detected in fish with high initial bacterial load *(23, 33)*. Maintaining low temperature during fish processing is one of the most important preventive measures. Thus, it is recommended that fish are cooled rapidly below 4°C on the vessel, after harvesting *(31)*. Low temperatures inhibit proteolysis and growth of aminogenic microorganisms; however, common cooling temperatures will prevent the growth of mesophillic aminogenic organisms, but not the psychrotolerant organisms. Some studies revealed that histamine formation could occur during storage of fish products at room temperature (anchovies packed in brine or oil *(23, 33)*). The level of histamine in fish products cannot be reduced by cooking, preservation, and freezing since histamine is heat stable *(34)*.

Serbia does not have a domestic marine fish industry, except for several facilities for processing marine fish. The aim of this research was to describe the case of massive histamine intoxication in Serbian children and to provide information on the presence of histamine in imported marine fish and fishery products available in the Serbian market.

MATERIALS AND METHODS

According to the U.S. Food and Drug Administration (FDA), a "lot" is identified as a specific amount produced in a unit of time or quantity in a manner that ensures its uniform character and quality within specified limits *(32)*. Each sample in this study represents one lot and consists of nine units of fish or fish products.

One sample (canned sardines from Tunisia) was collected in a kindergarten because of suspected massive histamine intoxication in children in January 2014. All other samples were collected during regular import control as part of an official veterinary control. From January 2013 to January 2015, 273 lots in total, including 2,457 fish and fishery product units, were analyzed: five different types of canned fish (tuna, sardine, salmon, mackerel, and herring), five different types of frozen fish (mackerel, sprat, tuna, salmon, and grouper), smoked fish (sprat), and quick frozen fish strips (made of different types of fish).

Countries that the samples originated from were Thailand (62.86% of canned tuna) and Croatia (52.06% of canned sardines), but some samples originated from Vietnam, Morocco, Spain, Poland, The Philippines, Russia, Estonia, and Brazil.

The presence of histamine in canned fish was analyzed by using enzyme-linked immunosorbent assay (ELISA) and the test kit HIS-E02 (Immunolab GmbH, Kassel, Germany). Histamine concentrations were determined according to the manufacturer's instructions. Subsamples of 10 g were extracted with 0.1 M hydrochloric acid. After dilution and derivatization, samples or standards containing derivatized histamine and an antibody directed against histamine were loaded into the wells of the microtiter plate. A histamine conjugate is bound on the surface of a microtiter plate, and immobilized and free histamine compete for the antibody binding sites. After incubation, washing, and removal of the unbound material, a peroxidase conjugate directed against the histamine antibody is added into the wells. After the second incubation, the plate is washed again and a substrate solution is added and then incubated, resulting in the development of a blue color. By adding a stop solution, the color turns yellow. The intensity of the yellow color is measured photometrically at 450 nm with a microplate photometer (Multiskan FC, Thermo Scientific, Shanghai, China) and is inversely proportional to the histamine concentration in the sample. RIDASOFT Win.net software (Z9999, R-Biopharm, Darmstadt, Germany) was used for the evaluation of the enzyme immunoassay. The laboratory detection limit for histamine determination is expressed as a blank sample value plus three standard deviations. The laboratory detection limit in fresh and canned fish was 2 and 10 mg/kg, respectively. Recovery of the method was 94.3%, based on histamine determinations in the reference material (lyophilized tuna muscle, T1134A-1/CM, Progetto, Trieste, Italy). The analytical quality of the ELISA was ensured by using reference material for the control sample and by participating in the proficiency testing scheme (canned fish sample, FAPAS 27110).

RESULTS AND DISCUSSION

Fish product–borne histamine intoxication outbreak. In the massive histamine intoxication case, 11 children manifested clinical symptoms of histamine intoxication immediately after consuming breakfast. A few hours later, the symptoms occurred in 17 more children. All the children were 2 to 5 years of age. The major and most prominent symptom included a pale red rash (urticaria); all children manifested numerous red spots on their faces, arms, and chest. The skin changes were red, ranged from 2 to 4 cm in diameter, and were edematous, yet without itching. Only six children manifested other symptoms of histamine intoxication, such as sweating, tachycardia, and headache. Literature data indicated that oral ingestion of histamine at concentrations below 50 mg/kg did not induce any symptoms; concentrations up to 150 mg/kg produced mild symptoms, manifested as moderate headaches and sweating; and histamine concentrations of 300 to 500 mg/kg led to the classical intoxication symptoms of rush, diarrhea, flushing, and headache *(3, 7, 20)*. A report from European Food Safety Authority *(7)* indicated that there is no straightforward dose-response relationship and symptoms never occur in 100% of patients. The manifestation of symptoms is associated with a range of factors, such as age, genetic predisposition, gastrointestinal diseases, histamine intolerance, and the use of certain drugs *(7, 18, 26)*. In our case, after serving of breakfast of canned sardines, 25% of the children were affected. Because the affected population included children and canned sardines are not typical for

TABLE 1. *Histamine content (presented in descending order) in canned sardines from Tunisia that were implicated in food poisoning, and other unsafe lots according to European Union criteria*

Product	Country of origin	Histamine content (mg/kg)								
		Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Unit 6	Unit 7	Unit 8	Unit 9
Canned sardines[a]	Tunisia	>500	>500	>500	476.9	461.6	446.8	350.0	173.5	144.8
Canned tuna	Thailand	233.9	196.0	178.3	146.9	121.0	74.8	70.8	41.0	35.3
Canned tuna	Thailand	437.3	274.7	268.6	207.4	183.1	158.6	152.6	135.2	75.5
Canned tuna	Thailand	>500	>500	>500	>500	473.5	390.0	271.6	161.3	144.2
Canned tuna	Thailand	>500	389.1	371.0	295.0	242.5	216.0	149.7	80.4	17.3
Canned tuna	Thailand	414.9	257.1	221.9	182.3	180.0	173.3	169.5	143.0	110.5
Canned sardines	Morocco	>500	252.9	155.2	90.8	71.3	55.5	47.7	41.7	38.6

[a] Canned sardines involved in massive histamine intoxication in Siberian children.

Serbian cuisine, we cannot precisely determine how many children consumed the sardines and in what amount. Analysis of nine units of the incriminated sardine cans revealed high histamine content in all cans (canned sardines from Tunisia, Table 1). Seven of the nine investigated units had histamine contents above 300 mg/kg, a concentration that leads to classical intoxication symptoms. In the affected children, the diagnosis was established based on anamnestic data, epidemiological data, and clinical symptoms and was confirmed by positive histamine finding in the incriminated foodstuff, i.e., the canned sardines. Symptomatic therapy was administered, and since there were no severe cases, antihistamine drugs were not given. The intoxication symptoms lasted 12 to 24 h, a time frame in accordance with literature data for histamine intoxication *(12),* and a full recovery without complications was recorded in all children. It is to be emphasized that lethal outcomes of histamine intoxication have not been recorded to date *(2, 7).*

Survey of imported fish and fish products in Serbia. Of 273 analyzed lots, 183 (67.03%) samples revealed histamine levels below the lower laboratory detection limit for canned and frozen fish of 10 and 2 mg/kg, respectively (Table 2). In the lots of frozen fish and fish products (74 samples), histamine levels were below laboratory detection limit (94.59%) or in the range between 10 and 50 mg/kg (5.41%). Similar levels were found by Park et al. *(22),* with the highest histamine value of 39.3 mg/kg in 30 frozen mackerel lots. The highest histamine level in 20 of the analyzed lots of canned mackerel measured in our research was 19.1 mg/kg, similar to the results obtained by Tsai et al. *(28),* who reported a maximum level of 24 mg/kg. Thus, neither the present study nor that of Tsai et al. *(28)* found unsafe samples of canned mackerel. Histamine content in our samples of canned tuna was unacceptable in 5.60% of the samples and very similar to the 4.76% value reported by Tsai et al. *(28).*

In 1995, the FDA *(29)* established a hazard analysis and critical control point program for the seafood industry and defined critical control points for analysis of marine fishery products. In scombroid species, the concentration of 50 mg/kg is considered to be the action level, and the regulatory limit has been established according to data based on numerous epidemics *(29).* According to the FDA *(30)* and Rogers and Staruszkiewicz *(24),* fish with histamine levels below 10 mg/kg are considered of good quality, 30 mg/kg indicates significant deterioration, and 50 mg/kg represents conclusive evidence of deterioration. In that respect, and according to Table 2, 93.40% of analyzed lots had histamine levels below the action level

TABLE 2. *Histamine content in fresh fish and fishery products from official border control in Serbia*

Product	No. of lots	No. (%) of lots with a histamine content (mg/kg) of:				No. (%) unacceptable
		<LOD[a]	10–50	50–100	>100[b]	
Canned tuna	125	68 (54.40)	43 (34.40)	5 (4.00)	2 (1.60)	7 (5.60)
Frozen tuna	2	2	0	0	0	0
Canned sardines	50	29 (58.00)	17 (34.00)	1 (2.00)	1 (2.00)	2 (4.00)
Canned mackerel	20	15 (75.00)	5 (25.00)	0	0	0
Frozen mackerel	21	17 (80.95)	4 (19.05)	0	0	0
Smoked sprat	1	0	1	0	0	0
Frozen sprat	14	14	0	0	0	0
Canned salmon	2	0	2	0	0	0
Frozen salmon	16	16	0	0	0	0
Canned herring	1	1	0	0	0	0
Frozen grouper	1	1	0	0	0	0
Quick frozen fish strips	20	20	0	0	0	0

[a] LOD, laboratory detection limit.
[b] Production lots in which histamine contents ranged from 100 to 200 mg/kg in at least two units.

of 50 mg/kg recommended by the FDA. The remaining 6.60% lots were canned products that were sterilized, so we conclude that these cans were produced from fish characterized by a significant level of deterioration (products originated from Thailand, Poland, and Croatia). The detected histamine levels suggested that the examined fishery products were produced from fish that were not fresh and should have been excluded from food processing for human consumption. The freshness of the fish is determined by fish species, duration and temperature of storage, and the level of microbiological defects. Fish spoilage is associated with protein degradation to peptides, free amino acids, and biogenic amines, all of which are useful indicators of raw material freshness *(22)*.

In Canada, Switzerland, and Brazil, the maximum permitted histamine level in fish and fishery products is 100 mg/kg *(9, 19)*. The legislative acts of the Republic of Serbia *(21)* are harmonized with the Directive from the European Union *(4)*. The examination of one lot includes testing of nine units. The permitted level implies that not more than two of nine sample units may contain between 100 and 200 mg/kg; however, none of the units may exceed the upper histamine limit of 200 mg/kg. Pursuant to these criteria, nine (3.29%) lots were declared unsafe for human consumption. All unsafe fish products in our study, i.e., seven canned tuna and two canned sardines, belonged to the family Scombridae. Official border inspection and prevention of the import of unsafe food are important measures for histamine control. Even though our research indicated that 96.71% of lots from imported fish and fishery products were safe for human consumption, there are always unscrupulous manufacturers whose unsafe products can cause human intoxication, as in our case of massive intoxication in Serbian children. Epidemiological research revealed that canned sardines from Tunisia involved in histamine intoxication of children did not undergo adequate control during import, because the lot numbers were irregular, and the cans had the same lot number for different production dates. No irregularity was noted regarding lot numbers during border inspection. Furthermore, in the samples of canned sardines that were produced on 24 September 2013, and that were controlled during border inspection, the histamine level was safe; intoxication of the children happened after consuming canned sardines produced on 22 September 2013. Additional control reveals six different production dates with the same lot number, and only sardines produced on 22 September 2013 had higher content of histamine.

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