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ASSESSMENT OF PARASITIC INVASIONS IN FISH MEAT ON THE CROATIAN MARKET

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SUMMARY

Croatian market is more and more supplied with imported fish, which are frequently infested with different parasites and their developmental stages. Since certain parasites present a potential risk to human health, constant veterinary-sanitary control of fish and fish products in distribution and sale is of utmost importance. This paper presents results of parasitological examinations of frozen hake ($n=143$) and redfish ($n=384$) imported in the Republic of Croatia. *Anisakis* spp. was found in both hake and redfish (66.4 % and 59.6%, respectively). Furthermore, in total 22.4% of hakes were infested with *Kudoa* spp. and 20.3 % of redfish with *Sphyrion lumpi*.

Key words: fish, parasites, food safety

INTRODUCTION

Parasites and their larval stages in fish meat can cause human diseases or organoleptical changes due to which fish are evaluated as hygienically inadequate for human consumption. Of utmost importance in evaluation of hygienic quality of fish are plerocercoid developmental stage of *Diphylobotrium latum*, metacercaria of *Opisthorchis felinus*, larvae of the nematode *Anisakis* spp. and development stages of *Myxosporea*, *Kudoa* spp. and *Henneguya zschokkei* (Kozačinski et al., 2002; Hadžiosmanović and Kozačinski, 2004).

Infestation of fish with larvae of *Anisakis* spp. is frequent in numerous fish species like codfish, hake, sardine, anchovy, salmon, red mullet, tuna and mackerel (Huang, 1988; Orecchia et al., 1989; Pereira Buena, 1992; Sanmartin et al., 1994, quotation Alonso-Gómez et al. 2004). This parasitic infection of fish is of great importance in public health, as it may lead to human diseases due to consumption of raw, or insufficiently heat-treated or preserved fish. Infection with live parasite larvae can be manifested by gastrointestinal symptoms or symptoms of other organ systems due to migration of the larvae through the intestinal wall (Romero et al., 1997; Louredo-Méndez et al., 1997; Amin et al., 2000; Matsuoka et al., 1994, Valls et al., 2005). Moreover, hypersensitivity reactions are possible (oedemas, urticaria, anaphylactic shock) to allergens of dead parasites (Alonso et al., 1997; Audicana et al., 2002). The studies have shown that larvae of *Anisakis* spp. migrate from visceral organs to muscles after the death of fish, so it is recommended to eviscerate the fish immediately after catch, as well as to remove the abdominal part of muscles (Wooten and Cann, 1982; FAO, 2001). In addition, *Anisakis* spp. larvae can be killed by heat treatment at 60°C or by freezing at -20°C for 60 hours (Živković et al., 1989,

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Živković et al., 1996; FAO, 2001; quot. Kozačinski et al., 2002).

In distinction from *Anisakis* spp, parasites of the genus *Kudoa* (*K. thyrsites*, *K. hystolitica*) do not present a direct risk to human health, but they may cause organoleptical changes that make usability of fish for human consumption questionable. After catch of fish infested with parasites, the *post-mortem* autolysis caused by the parasite proteolytic enzymes occurs (Dykova et al., 2002). Losses caused by infestation with this parasite can be direct, due to inferior value of fish meat, or indirect, because of the consumer's perception that fish from a particular area are of inferior quality. Infestation with the ectoparasite *Sphyrion lumpi* of the genus *Copepoda* also results in organoleptical changes of fish meat in the form of tissue reactions on the predilection sites of infection (thinner parts of skin, anal area, damaged skin). Parasite finding, either whole or its parts in muscles (head and thorax), reduces fish quality making it often useless for human consumption.

The above mentioned noxious effects of parasites and their developmental stages are the reason for undertaking systematic measures of control and inspection in both the national and international trade of fish and fish products. In addition to a rich offer of domestic sea fish on the Croatian market, there is also a lot of imported fish of questionable quality. Therefore, the aim of this study was to determine the level of parasite infestation in the imported frozen hake and redfish.

MATERIALS AND METHODS

Parasitological examination was done on samples of frozen hake and redfish sampled during the routine veterinary control at the time of import. Samples were taken from 4 consignments of imported fish.

In frozen blocks of redfish (n=2) there were 112, 272 fish respectively, and in hake blocks (n=2) 105, 38 fish respectively. After defrosting, samples of redfish and hake were examined for the presence of nematodes in the abdominal cavity and muscles. Presence of parasites in muscles was checked by digestion method: 200 g of fish tissue was added to 750 ml of warm pepsin solution. Beaker was placed in a 37°C shaking water bath (low speed/15 min).

The pH of sample was adjusted to 2 with 6 N HCl, and shaken for 24 h. The digested material was poured through a sieve into a suitable container. The material passed through the sieve was examined by transferring it to a clamped funnel where it was allowed to settle for 1 h, and then the sediment was drained into a beaker (Government of Canada, 1995). The parasite morphology was examined under microscope. After filleting, the fish meat was examined for the presence of pseudocysts of *Myxosporea* and redfish also for the ectoparasite presence on the skin and deep in muscles. Pseudocyst material was smeared on a slide, dyed with brilliant-green and examined under microscope.

RESULTS AND DISCUSSION

Table 1 presents total results of parasitological examination of frozen redfish and hake. In the first sample of redfish (n=112), visceral remnants were found, and in the abdominal cavity of 47% of redfish were found dead, yellowish-white spiral larvae of the species *Anisakis*. Larvae count per fish ranged from 4 to 10. On the skin surface of 4.5% of fish, 5-10 cm long, partly damaged ectoparasites of the species *Sphyrion lumpi* were found embedded in the fish muscles. Greyish-brown formations of bean size were found in muscles of 21% of fish, in which only the parasite cephalothorax was found. In the second redfish sample (n=272), incomplete evisceration was also noted, and in the abdominal cavity of 65% of redfish were found dead spiral larvae of the species *Anisakis*. Number of larvae per fish ranged from 3 to 10. On the skin surface of 15% of redfish, 15 cm long, white ectoparasites of the species *Sphyrion lumpi* were found embedded in muscles. In muscles of 5% of fish were detected limited tissue reactions of grey-brown colour and bean size, containing only the parasite cephalothorax. In the first hake sample (n=105), visceral remnants were also found, and in the abdominal cavity and muscles (artificial digestion method) the *Anisakis* larvae were found in 90.5% of fish. In the second hake sample (n=38) in 80.4% of filleted fish were found oval, milky-white to brown cystic formations of 1-5 mm, filled with greyish-yellow pulpy mass. Microscopic examination revealed that these formations were Myxozoa spores of the

▼ **Table 1** Level of parasite infestation of imported fish▼ **Tablica 1.** Stupanj invazije parazitima ribe iz uvoza

Fish species Vrsta ribe	Quantity/N° Broj pretraženih riba	Parasite species Vrsta parazita		
		Anisakis	Kudoa	Sphyrion lumpi
Redfish Škarpina	384	59.6%	-	20.3%
Hake Oslić	143	66.4%	22.4%	-

species *Kudoa*. Cumulatively shown, *Anisakis* spp. was found in 66.4% of hakes and 59.6 % of redfish. Furthermore, 22.4 % of hakes were infested with the *Kudoa* spp, and 20.3% of redfish with *Sphyrion lumpi*.

It is important to note that different countries have different national regulations, or they give non-mandatory guidance, for the control of nematodes as a human health hazard in fish products. Importers and exporters should be aware of the requirements of the current legislation. Within the European Union, lightly cured products, such as marinades and smoked fish that are intended for consumption without further cooking, must be frozen "at the temperature not exceeding -20°C in all parts of the product for not less than 24 hours", in addition to the curing process intended. In good practice, this should be interpreted as quick freezing to a minimum of -20°C at the thermal centre, followed by storage at or below -20°C for at least 24 h (FAO, 2001).

Assessment of food health safety in Croatia is regulated by the Food Law (NN 117/2003) and by the By-law on methods of veterinary-sanitary control of animals before slaughter and food products of animal origin (NN 53/91). According to the Food law, food containing tissue parasites dangerous to human health, as well as other parasites exceeding permissible levels is considered to present a risk to human health. Moreover, food is inadequate for human consumption if sensor properties of food are altered due to physical, chemical, microbiological or other processes. According to the By-law on veterinary-sanitary control methods of animals before slaughter and animal food products (NN 53/91), the

frozen fish are considered as hygienically unacceptable for human consumption if their meat contains pathogenic developmental stages of *Diphylobotrium latum*, *Opisthorchis felineus* and *Anisakis* spp or other parasites and their developmental stages. The same applies to apathogenic parasites, which cause organoleptic changes of fish. With regard to the mentioned regulations, the examined samples of redfish and hake were found not to be health safe because of the finding of parasites noxious to human health, as well as because of organoleptical alterations induced by apathogenic parasites.

PROŠIRENI SAŽETAK NALAZ PARAZITARNIH INVAZIJA U MESU RIBE S HRVATSKOG TRŽIŠTA

Paraziti i njihovi larvalni oblici u mesu riba mogu uzrokovati oboljenja ljudi ili pak organoleptičke promjene uslijed kojih se riba ocjenjuje higijenski neispravnom za prehranu ljudi. U tom smislu najveće značenje u ocjeni higijenske ispravnosti ribe imaju plerocerkoidni razvojni stadij *Diphylobotrium latum*, metacerkarija *Opisthorchis felineus*, larve nematoda *Anisakis* spp te razvojni oblici *Myxosporea*, *Kudoa* spp i *Henneguya zschokkei* te invazije ektoparazitom *Sphyrion lumpi* iz roda *Copepoda* (Kožačinski i sur., 2002 ; Hadžiosmanović i Kožačinski, 2004). Navedene činjenice o štetnosti parazita i njihovih razvojnih oblika razlogom su provođenju sustavnih mjera kontrole i nadzora u domaćem i međunarodnom prometu ribom i ribljim proizvodima. Budući da je na hrvatskom tržištu, pored bogate ponude domaće morske ribe, svoje mjesto našla i riba iz uvoza koja je često upitne kakvoće, cilj ovog rada bio je utvrditi stupanj invadiranosti parazitima smrznutih oslića i škarpina podrijetlom iz uvoza.

Parazitološka pretraga obavljena je na uzorcima

smrznutih oslića i škarpi uzorkovanih pri veterinarskoj kontroli i nadzoru kod uvoza. Uzorci su potjecali iz 4 pošiljke proizvoda podrijetlom iz uvoza (smrznuti blokovi škarpi i oslića). Po odmrzavanju uzorci škarpi i oslića pregledani su na prisutnost nematoda u trbušnoj šupljini i mišićju. Prisutnost nematoda u mišićju provjerena je postupkom umjetne probave (Government of Canada, 1995). Morfologija nematoda provjerena je mikroskopski. Nakon filetiranja mišićje ribe je pretraženo na prisutnost pseudocista miksosporidija, a uzorci škarpi i na prisutnost ektoparazita na koži i u dubini mišićja. Sadržaj pseudocista je razmazan na predmetnicu, obojan briljantnim zelenilom i pregledan mikroskopski.

U tablici 1 prikazani su skupni rezultati parazitološke pretrage smrznutih škarpi i oslića. U prvom uzorku škarpi (n=112) utvrđeni su ostaci utrobe, a u 47 % škarpi pronađene su uginule spiralno zavijene larve *Anisakis* spp žučkasto bijele boje koje su se nalazile slobodni u trbušnoj šupljini. Broj larvi po ribi kretao se od 4 do 10. Na površini kože 4,5 % riba nađeni su 5-10 cm dugački, dijelom oštećeni ektoparaziti vrste *Sphyrion lumpi*, ubušeni u mišićje riba. U mišićju 21 % riba utvrđene su sivosmeđe tvorbe veličine graha u kojima je pronađen samo cefalotoraks parazita. U drugom uzorku škarpi (n=272) također je utvrđena nepotpuna evisceracija svih riba, a u trbušnoj šupljini 65% škarpi utvrđene su uginule spiralno zavijene larve *Anisakis* spp. Broj larvi po ribi kretao se od 3 do 10. Na površini kože 15 % škarpi nađeni su 15 cm dugački, bijeli ektoparaziti vrste *Sphyrion lumpi*, ubušeni u mišićje ribe, dok su u mišićju 5 % riba utvrđene ograničene reakcije tkiva sivosmeđe boje veličine graha u kojima je pronađen samo cefalotoraks parazita. U prvom uzorku oslića (n=105) također su uočeni ostaci utrobe, a u trbušnoj šupljini i mišićju (umjetnom probavom) 90.5 % riba utvrđene su larve *Anisakis* spp. U drugom uzorku oslića (n=38) u ukupno 80.4 % riba nakon filetiranja su uočene ovalne, mliječnobijele do smeđe cistične tvorbe veličine 1 do 5 mm ispunjene sivo-žučkastom kašastom masom. Mikroskopskom pretragom utvrđeno je da se radi o sporama *Myxozoa*, *Kudoa* spp.

Ocjena zdravstvene ispravnosti hrane u R Hrvatskoj regulirana je Zakonom o hrani (NN 117/2003) te Pravilnikom o načinu obavljanja veterinarsko-sanitarnog pregleda životinja prije klanja i proizvoda životinjskog podrijetla (NN 53/91). Prema Zakonu o hrani štetnom za zdravlje ljudi smatra se hrana koja sadrži tkivne parazite opasne po zdravlje ljudi kao i druge tkivne parazite iznad dopuštenih količina. Nadalje, hrana je neprikladna za ljudsku konzumaciju ukoliko su senzorna svojstva hrane zbog fizikalnih, kemijskih, mikrobioloških ili drugih procesa toliko izmijenjena da hrana nije prikladna za prehranu ljudi.

Pravilnikom o načinu obavljanja veterinarsko-sanitarnog pregleda životinja prije klanja i proizvoda životinjskog podrijetla (NN 53/91) u dijelu koji se odnosi na higijensku ispravnost zamrznute ribe, propisano je da se higijenski neispravnom za javnu potrošnju smatra zamrznuta riba u čijem su mesu pronađene razvojni oblici *Diphylobotrium latum*, *Opistorchis felinus*, *Anisakis* spp ili drugi paraziti i njihovi razvojni stadiji patogeni za ljude, kao i nepatogeni paraziti koji su uzrokovali organoleptičke promjene ribe. U duhu navedenih odredbi, pretraženi uzorci škarpi i oslića proglašeni su zdravstveno neispravnima, uslijed pronađenih parazita štetnih po zdravlje ljudi, kao i organoleptičkih promjena uzrokovanih nepatogenim parazitima.

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EKOLOŠKI UZGOJ SVINJA

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SAŽETAK

U Republici Hrvatskoj postoje svi prirodni uvjeti za uspješnim uzgojem svinja na ekološki prihvatljiv način. Pritom treba poštivati zakonom određene zahtjeve, a odnose se na: opće uvjete, zootehničke postupke, smještaj i držanje te hranidbu i zdravstvenu zaštitu svinja. Pri organizaciji smještaja svinje se mogu držati u zatvorenim prostorima ili na otvorenom. Kod držanja svinja u zatvorenom razlikujemo nekoliko funkcionalnih prostora, koji se dijele na prostor za baleganje, ležanje, hranjenje i izlaz za slobodno kretanje. Držanje na otvorenom može biti organizirano za sve kategorije svinja izuzev krmača neposredno pred prasenje i prasadi u prvom periodu života. Pritom treba izbjegavati nepropusna i močvarna tla, jer prekomjerna vlažnost može biti

uzrokom zdravstvenih poremetnji. U cilju zaštite od sunca i nepovoljnih vremenskih prilika, na otvorenim površinama postavlja se dovoljan broj kućica, koje veličinom odgovaraju pojedinim dobnim skupinama svinja.

Ključne riječi: svinje, ekološka proizvodnja, zakonska regulativa

UVOD

Ekološki uzgoj svinja temelji se na držanju pojedinih kategorija svinja u što prirodnijem okolišu. Takav način uzgoja omogućuje humaniji odnos prema životinjama radi zadovoljenja njihove dobrobiti i zapravo je alternativa konvencionalnoj svinjogojskoj proizvodnji. Osim toga, takav pristup držanja svinja omogućuje očuvanje okoliša, jer se sprječava proizvodnja

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