

SURVEILLANCE OF FISH DISEASES IN SERBIA

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NADZOR BOLESTI RIBA U SRBIJI

Apstrakt

U Srbiji se vrši program nadzora virusnih i bakterijskih bolesti na osnovu Programa mera zdravstvene zaštite životinja, u skladu sa propisima Evropske Unije, i to: virusne hemoragične septikemije (VHS) i zarazna hematopoezne nekroze (IHN), zarazne nekroze pankreasa (IPN), prolečne viremije šarana (SVC) i renibakterioze (BKD). Cilj programa praćenja i nadzora bolesti riba je dobijanje i održavanje statusa slobode od bolesti, iskorjenjivanje ili sprečavanje širenja bolesti. Primarna ciljna populacija u programu nadzora su kalifornijska pastrmka i šaran.

U Srbiji postoji nacionalno zakonodavstvo kao osnov za nadzora i kontrolu bolesti riba, kao i lista bolesti obaveznih za prijavljivanje. Pored nacionalnog zakonodavstva, prihvaćeni su i principi navedeni u Direktivi 2006/88/EC, koji se odnose na zahteve za kontrolu zdravlje riba u akvakulturi i njihovih proizvoda. Praćenje i nadzor virusnih bolesti vrše se na osnovu postupaka ispitivanja datih u Odluci Komisije 2001/183/EC, a za bakterijske bolesti, koriste se standardne dijagnostičke procedure.

Klinički pregledi na ribnjacima vrše se dva puta godišnje i uzimaju se uzorci za virološka i bakteriološka ispitivanja, u svrhu dokumentovanja odsustva bolesti. Postupak ispitivanja, dat u Priručniku OIE za dijagnostiku bolesti riba, osnova je za ispitivanja. Uzorci svih kategorija riba iz 56 šaranskih i 52 pastrmska ribnjaka se godišnje ispituju na prisustvo bolesti. Za virusološka ispitivanja su korišćeni homogenati bubrega, slezine, jetre i škrge. Pulirani parenhimatozni organi i škrge su homogenizovani i centrifugirani na 2500 x g, 20 minuta. Za izolaciju, supernatanti su inokulirani na 24 sata stare kulture EPC i BF-2 ćelijskih linija. Inokulisane kulture su inkubirane na 15 - 20 °C, tokom 7 dana i svakodnevno su posmatrane na pojavu citopatogenog efekta. Identifikacija virusa je vršena PCR, ELISA testom i testom fluorescentnih antitela. Kao materijal za PCR je uziman homogenat organa i prva ili druga

pasaja odgovarajuće ćelijske linije. PCR produkti su sekvencirani direktno, pomoću Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, SAD) i ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). Dobijene sekvence su analizirane pomoću Sequencing Analysis Software 5.1 (Applied Biosystems). Na osnovu rezultata nadzora, Srbija se smatra slobodnom od VHS, IHN i KHV. Najveći problem predstavlja zarazna nekroza pankreasa. Renibakterioza je prisutna na određenom broju ribnjaka.

Ključne reči: nadzor, bolesti riba

Keywords: surveillance, fish diseases

INTRODUCTION

On a global scale, fish and fishery products are the main food supply for human beings. It is widely known that the supplies of fish from traditional fisheries are more or less constant and that the shortage in fish and fish products has to be met by aquaculture. The intensive aquaculture is often characterized by high density of fish, poor water quality, accumulation of pathogens in the production systems and in the environment. As a result, most populations of fish from intensive rearing systems are characterized by chronic stress. Stress leads to increased susceptibility to disease, and prevalence of disease depends on the interaction between fish pathogens and the environment (Jeremić, 2003). The appearance and development of fish diseases is a consequence of the interaction of pathogen, host and environment. Also, international trade of live fish and their products is a major hidden cause of many outbreaks. Damages caused by the disease significantly delay the development of fisheries and prevent its transition to modern forms of intensive aquaculture. Many diseases affecting farmed fish also represents a threat to natural fish populations (Thoessen, 1994). The relatively small number of pathogenic bacteria is responsible for major economic losses in cultured fish (Toranzo et al., 2005). In addition to the release of active substances in aquatic ecosystems, the usual therapeutic interventions in aquaculture can lead to antibiotic resistance in bacterial pathogens of fish, but also in other bacteria present in the environment (Alderman and Hastings, 1998).

MATERIAL AND METHODS

Samples of all fish categories from 56 carp and 52 trout farms were examined annually for the presence of certain viral and bacterial diseases. Clinical examination and selection of samples for laboratory was done on the fish farms. For virus isolation, homogenates of kidney, spleen, liver and gills were used. Pools of parenchymatous organs and gills were homogenized with MEM and centrifuged at 2500 x g, 20 minutes. Supernatants were inoculated at 24 hours old culture of EPC, CCB and BF-2 cell lines. Inoculated cultures were incubated at 15 - 20°C for 7 days and observed daily by the appearance of cytopathic effect. For virus identification, ELISA, FAT, PCR, RT-PCR and sequencing were performed. As a material for PCR extracted organ homogenates and the first or second passage of the appropriate cell lines were used. DNA was extracted using a DNA mini kit according to the manufacturer (QIAGEN, USA). RNA was extracted using RNA mini kit according to the manufacturer (QIAGEN, USA). PCR products were sequenced directly using Big Dye Ter-

minator v1.1 Cycle Sequencing Kit (Applied Biosystems, USA) and ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). The obtained sequences were analyzed using Sequencing Analysis Software 5.1 (Applied Biosystems).

RESULTS AND DISCUSSION

The fish farming industry is based on cold water species, rainbow trout (*Onchorhynchus mykiss*), brown trout (*Salmo trutta m. fario*) and warm water species, common carp (*Cyprinus carpio*), grass carp (*Ctenopharingodon idella*), silver carp (*Aristichthys nobilis*), bighead carp (*Hypophthalmichthys molitrix*), catfish (*Silurus glanis*), pike (*Esox lucius*), and pike perch (*Stizostedion lucioperca*). In Table 1 an overview over the farmed fish species as well as the size and production of these species are given.

Over the last decade, several emerging or serious diseases in fish have been diagnosed in farmed and feral populations, creating large problems in the fish farming industry and thus being the subject of surveillance and monitoring programs. Differences between infectious diseases in fish and those of terrestrial animals mean that the approach to the problems and the eradication efforts differ as the diseases may spread effectively through the flowing water (Håstein et al., 1999).

Table 1. Number of fish farms, species and production in Serbia

No. of farms	Species	Total production
108	rainbow trout (<i>Onchorhynchus mykiss</i>) brown trout (<i>Salmotrutta m. fario</i>) common carp (<i>Cyprinus carpio</i>) grass carp (<i>Ctenopharingodon idella</i>) silver carp (<i>Aristichthys nobilis</i>) bighead carp (<i>Hypophthalmichthys molitrix</i>) catfish (<i>Silurus glanis</i>) pike (<i>Esox lucius</i>) pike perch (<i>Stizostedion lucioperca</i>)	14.000 tons

In the following, the basis for and the results of monitoring and surveillance programs were described (Tab.2), as well as measures established for some of the diseases considered to be of major concern.

Table 2. Diseases and target species under surveillance

Disease	Susceptible species
IPN	Salmonids
VHS	Salmonids
IHN	Salmonids
SVC	Cyprinids
KHV	Carp & Koi carp
BKD	Salmonids

Serbia has a national legislation as a basis for aquatic animals' disease surveillance and control, as well as regulations listing notifiable diseases of concern. In addition to the national legislation, the principles laid down in the Council Directive 2006/88/EC in regards to animal health requirements for aquaculture animals and products thereof were accepted. Monitoring and surveillance of fish viral diseases have mainly been based on the testing procedures given in the Commission Decision 2001/183/EC while for the bacterial diseases, standard diagnostic procedures has been used for screening purposes.

Serbia runs a surveillance program for viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) based on EU regulations. Monitoring program for infectious pancreatic necrosis (IPN), spring viraemia of carp (SVC) and bacterial kidney disease (BKD) takes place in Serbia on a national level. The aim of the monitoring and surveillance programs for fish diseases is to document and maintain freedom of disease, either to eradicate a disease or to keep it under control within certain bounds.. The main target population for the monitoring and surveillance programs are rainbow trout and common carp. The size of the target populations is given in Table 1. All types of farms are included in the survey such as hatcheries, brood stock farms as well as grow out farms. The diseases under surveillance and the target species for the disease in question are shown in Table 2.

The basis for the surveillance and monitoring programs is partly based on EU regulations, OIE criteria or criteria derived from the national legislation. The participation is compulsory as regards approval and maintenance of disease free status. For the sampling of fish for surveillance the responsible authority is the district veterinary inspector in coordination with the local veterinary institute and the national reference laboratory.

Fish farms are inspected clinically biannually and samples for virological examinations are collected from the fish farms each year to document freedom for IHN, IPN and VHS. The examination procedures given in the OIE Diagnostic Manual for Aquatic Animal Diseases are the basis for examinations as regards KHV, BKD, and SVC.

The veterinary authorities are responsible for the implementation of measures that will be used in order to control a given notifiable disease. The implementation involves both central and regional veterinary officers. If disease is diagnosed, stamping out procedures followed by cleaning, disinfection and fallowing will be carried out. Prevention may be achieved by avoiding introduction of disease free eggs and/or fish into disease free farms, as well as using protected water supply (e.g. spring-, borehole water). An infected farm may restock after fallowing if no signs of infection appear after a sanitation program has been carried out. In Serbia affected farm will have to pay themselves for any measures imposed by the authorities for the time being, because no compensation is granted. Health certificates and/or transportation documents is needed in connection with deliveries of live fish for stocking into grow out farms and restocking into rivers. The record of findings are kept by the responsible authorities, both regionally and centrally. The diagnostic laboratories also keep the necessary documentation on the examinations carried out. Furthermore, all farms have to keep records on events in the farms that can be requested by Competent Authority.

CONCLUSIONS

Serbia is considered to be free from VHS, IHN and KHV. Infectious pancreatic necrosis is considered to be the main viral disease problem. BKD has been reported but the level appear to be relatively low. Serbia have established appropriate surveillance and monito-

ring for fish diseases of concern to the fish farming industry. Due to these systems and good management practices, the fish disease situation is generally good compared to other countries.

ACKNOWLEDGMENT

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia as part of projects number 31011 and 31075.

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