INFECTIOUS PANCREATIC NECROSIS (IPN) MIXED AND ASSOCIATED INFECTIONS IN THE TROUT AQUACULTURE IN BULGARIA

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ABSTRACT

Infectious diseases in aquaculture rarely occur on their own. Coinfections - mixed or associated occur commonly. Infectious pancreatic necrosis is a viral infection that occurs in juvenile trout. The IPN virus is very resistant, the transmission is vertical and horizontal and the sustainability out of the host is huge. In Bulgaria, mixed infections involving fish viruses are not enough studied. The global data is also insufficient. Trout aquaculture in our geographical settings often suffer from slight parasitic invasions and transitory bacterial infections. Notably, when it is established under certain factors, some of them become epizootic. The world's largest burden is onto viral infections. They occurs rarely but with incorrigible damages. For the past 8 years in Bulgaria only the IPN virus – in seemingly acute form, has been detected. This work presents clinical cases identified in the NRL BRMMP and a summary of the current global experience of the IPN coinfections – mixed and associated.

Key words: IPN, IPNV, viral infections, mixed infections, associated infections, coinfections, trout aquaculture.

Introduction

Infectious diseases in aquaculture normally run independently. More common are mixed or associated with other pathogens. Diagnosis is focused ordinarily on the establishment of the leading infectious agent, and insufficient attention is paid to the concomitant microfauna and microflora – pathogenic or useful (1, 2). In the infectious pathology of aquatic animals, the term „coinfections" is used when more than one infectious agent is involved in a disease as a synonym for a mixed infection; associated infection; competitive infection; multiple infection; double infection; secondary infection; superinfection (2).

Worldwide fish coinfection data is scarce. McLoughlin shows conditional percentage distribution (Fig. 1) of diseases caused by viruses, bacteria, parasites and fungi in aquaculture (3):

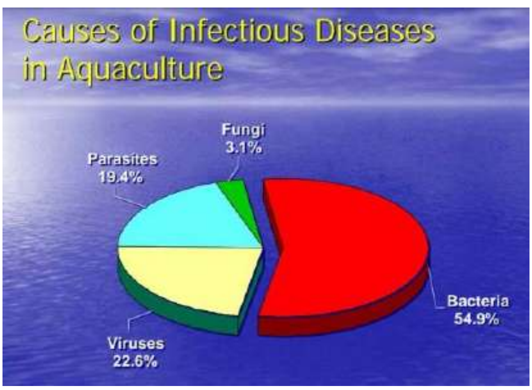

Figure1: Percentage distribution of diseases in aquaculture caused by different classes of pathogens (by Dr Marian McLoughlin, Aq. Vet. Services, Belfast, 2014).

The virus of infectious pancreatic necrosis (IPNV) is widespread. Affects salmon species. It is characterized by a horizontal and vertical transmission and a high environmental resistance. Vertical transmission is carried out by intraovarial carrier of the virus from one generation to another with fertilized eggs (4).

The virus causes death in freshwater trout weighing 0.1–10 g. Mortality can reach 90%. A window of susceptibility is also present at 80–100 g. Survived fish are the source of infection through excreta. Little is known about the natural way of penetration and the onset of infection. The carrier is latent, asymptomatic (5).

In Bulgaria coinfections involving fish viruses have been poorly studied. In the last 8 years only IPNV has been discovered in salmon farms in Bulgaria. Trout aquaculture grown in our geographical conditions most often suffer from unstable parasitic invasions or transient bacterial infections. If the technology group put in high density or another stress, a virulence of a weak pathogen may become epizootic.

The aim of this work are the clinical cases identified in the NRL BRMDR for the period 2011–2017 and a short view of fish coinfections in other countries.

Materials and methods

Results from laboratory tests carried out under the National Program of Surveillance (NPS) or from the samples sent mandatory by different fish farms on the territory of Bulgaria were used. All specimens were necessarily virologically tested and subjected to an external examination, pathoanatomic examination, parasitological status and bacteriological test in the primary inspection of the samples. 699 specimens of salmon trout from different technology groups, ranging from 104 farms, were examined.

For virological testing, standard laboratory methods were applied using the Office International des Epizooties (OIE) Manual of Diagnostic Methods on Aquatic Animal Diseases. A parasitological study was performed with a Zeiss Axioscope 2 plus light microscope. Microbiological testing was performed on indications resulting from the pathoanatomic finding.

Results and discussion

During the indicated period, 32 cases of IPN in salmon aquaculture and 2 IPNV carriers in fertilized eggs were detected. These 32 cases includes 5 associated with the virus and a high degree of invasion of Costia parasites and 1 of a mixed infection with the bacterium *Flavibacterium psychrophilum*. No viral coinfections with viral haemorrhagic septicemia (VHS) or Infectious haematopoietic necrosis (IHN) have been detected.

There have been no cases and samples from protected teritory or national parks, sport fishing objects or from natural trout species.

The data are shown in a Table 1.

Table 1: Samples of salmon fish obtained for study in the period 2011–2017

Species	Samples	IPNV	IPNV + bacteria	IPNV + ectopara-sites
Rainbow trout Brown trout	689	26	1	5
Brook trout	7	0	0	0
Coho salmon	3	0	0	0

Mixed and associated infections (coinfections) with IPNV involvement have been identified in many cases worldwide (2).

Homologous infections („virus-virus“)

IPNV occurs in co-infection with viral diseases such as: IHNV; VHSV; infectious salmon anemia (ISAV) and Salmon alphavirus infection (SAV).

The most explored interaction is between IPNV-IHNV. Double infection occurs intracutaneously and leads to a reduction in the IHNV antigen titer, whereas the IPNV titer remains the same. The mechanism of interaction is still not well understood, but Kim et al. (6) found that IPNV induces intracellular synthesis of an interferon antagonist to IHNV. Coinfections with the same mechanism are IPNV-ISAV and SAV-IPNV (7). The antagonistic effect of the aquabirnaviruses on VHS was demonstrated as obligatory (8). Maj-Paluch et al. in 2018 have found that the presence of the IPN virus reduces the proliferation of other viruses in the internal organs or in the specific salmon cell cultures (7).

Heterologous infections (IPNV and bacteria, parasites, fungi)

Infection with IPNV and *F. psychrophilum* occurs with synergism and potentiation of flavobacteriosis, at the appropriate age and size of the fish, the outbreak of viral infection is higher in mortality than in monoinfection (9). In the association of IPNV and *Vibrio salmonicida*, the action is synergistic, but the disease develops with lower mortality (10). Attempts to simultaneously artificially infect salmon with IPNV and *Aeromonas salmonicida* or *Yersinia ruckeri* show no significant interaction or change in the course of the leading infection but the study was conducted with vaccine strains (11, 12). IPNV was isolated from adult salmon fish with a bacterial kidney disease (BKD) clinic, caused of *Renibacterium salmoninarum* (13).

Mass parasitic invasions associated with viral infection potentiate the virus infection. Ectoparasites mechanically open an entry door to penetrate viruses. Although the appropriate environmental factors and a high degree of parasitic invasion onto susceptible fish group leads decreasing at immunity level. The virus is then activated even at a low titer and runs rapidly with high-mortality (14).

Diagnosis of IPN is based on a history, clinical signs (Fig. 2) and laboratory research. Differential diagnosis is impossible with a clinic and a history only. Laboratory practice demonstrated no clear picture from macroscopic findings.

A **B**

Figure 2: Clinical signs of IPN (non-specific) – ascit, exophthalm, black body trunk, open gills; A – ***post mortem*****; B –** ***in situ.***

When identifying the isolates by immunofluorescence method (IF) and in the presence of a second virus, the affected cells of the macro-organism can simultaneously detect the two viruses (16).

It is approved that the IPNV is widespread. For the purposes of viral investigation OIE recommends that the inoculum from samples should be pre-treated with IPNV antisera to suppress interference and facilitate the cultivation of a second tentative virus (17). If detection by molecular methods is conduct, the amplification is happens even at low titers but there is no evidence of a living virus (16).

The cumulative mortality rate for dual mixed viral infection is about 50% lower than for monoinfection with IPNV or another virus that may co-occur (18). This is used to create vaccines. A selection of fish based on gene-carriers of cadherin-induced resistance to IPNV has been developed with success in Norway. The breeding of technology groups of salmon fish carrying this gene allows 100% resistance to IPNV but this puts unresolved questions about the opening of a niche with no barrier for other viral pathogens (19).

Particular interest in aquaculture in recent years is the use of natural prebiotics and probiotics. As a bacterial vaccine against IPN in salmon aquaculture recombinant strains of *Lactobacillus casei* is used (20).

Until the 1990s, VHS covered over 38% of local salmon farms (21). It is logical to suppose that VHSV should be persist in part of the natural ponds where susceptible aquaculture species are still being grown but over the past 10 years the VHS virus has not been established.

Conclusion

In this article for the first time in Bulgaria attention is paid to the possibility of a complex manifestation of two or more pathogens (coinfection) on an aquatic susceptible macroorganism.

In the salmon aquaculture, cases of IPNV associated parasite infection and mixed infection with bacteria have been observed in Bulgaria.

Perhaps the lack of clinical information and mortality due to viral infection in fish is due to the already widespread prevalence of IPNV and it's proven antagonism in interactions with other fish viruses.

Probably, IPNV – operating with other fish pathogens, serve as a modulator of infectious process at the ecosystem. It's accomplishe through antagonism and synergy with the microfouna of the natural environment.

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