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The occurrence and prevention of Infectious Pancreatic Necrosis Virus (IPNV) in rainbow trout fish farms: the importance of improved sanitary practices

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Abstract

The viral diseases of rainbow trout are of worldwide concern including infectious pancreatic necrosis (IPN). On this study good sanitary practices are applied to prevent outbreaks and minimize losses caused from IPN disease. To highlight importance of improvements on sanitary practices in IPN prevention were compared results from two studies, first conducted in year 2006-2009 with results from one year later study conducted in 2010. In year 2010, samples of organs are collected from 260 fish and pooled in 43 samples, taken in 18 trout farms. Samples are analyzed with RT-PCR method. In the second study the IPN virus was detected in 6 from 43 pools (14%) showing higher infection rate comparing to first study (11.5%) and in 4 from 18 fish farms (22.2%) showing lower infection rate than in first study with 7 from 13 fish farms (53.8%). Results indicated that application of good sanitary practices reduces IPNV presence in fish, but not in fish farms where infection already is established and do not apply preventive measures. The purpose of this paper is to describe occurrence of IPNV and application of good sanitary practices in order to reduce disease outbreaks in rainbow trout fish farming.

Key words: ***Fish diseases, rainbow trout, sanitary practices, infectious pancreatic necrosis.***

1. Introduction

Viral diseases outbreaks are big concern in fish farming because of high mortality and no medication is possible. Infectious pancreatic necrosis virus (IPNV) is the causal agent of a highly contagious disease of young hatchery-reared rainbow trout. Infectious pancreatic necrosis virus (IPNV) is a member of the Genus *Aquabirnavirus*, family *Birnaviridae* [8]. The disease can be transmitted horizontally as well as vertically through ovarian and seminal fluids. The IPNV disease is initially detected in North America [7] with mortality rate ranging from 70-100% in fingerlings and early juvenile fish, usually following stressing conditions [1].

The most common approach to prevent viral diseases is to prevent contact of the pathogen with the host. Preventing the introduction of a pathogen into a new stock of fish has been accomplished mainly by implementing legislation to prevent transport of infected fish into uninfected areas, destroy fish already infected and the hatchery disinfection and restocking fish free of specific pathogens. Special care should be with IPNV positive eggs, were vertical transmission has been shown to occur despite comprehensive surface disinfection of eggs, as the virus may be carried internally in eggs [5, 11]. Even more difficult to control IPNV infection is because the virus has been found in wild fish and shellfish as well. The IPNV presence in wild brown trout in Kosova rivers has been reported [6]. The virus can persist in the environment and even in the guts of piscivorous birds [4]. In previous study on samples taken from farmed rainbow trout in Kosovo [8] IPNV positive

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pools were identified on level of 11.5% (13 of 113 pools), originating from seven IPNV positive farms (7 from 13) or 53.8%.

The aim of study is to show occurrence of IPNV and highlight the importance of application of good sanitary practices in reducing IPN outbreaks in rainbow trout fish farms and contributes on further improvements of disease prevention and control strategy.

2. Materials and methods

2.1. Fish sampling.

Samples were collected from fish internal organs. Sections of liver, spleen, kidney from each fish were taken and pooled (five fish per pool) in a small plastic tube. This was carried out on March 2010. A total of 43 tissue pools originating from 18 trout farms were sampled. The samples were kept in isopropanol 98%.

2.2. RNA extraction, reverse transcription and cDNA amplification. Total RNA was extracted from 30 mg fish tissue, from pooled organs (liver, kidney, spleen) using RNasy Mini Handbook (Qiagen).

2.3. RT-PCR mixture (Abgene) contained: Reverse IT Master mix (Thermoprime plus DNA polymerase 1.25 U/50 µL, optimized reaction buffer dNTP 0.2 mM, $MgCl_2$ 1.5 mM, RTase Blend (50 U/µL) including RNase inhibitor), 1 µL (50 pmol/µL) of sense and antisense primer, 1µL reverse IT Blend, 19 µL distilled water and 3 µL from sample.

2.4. Primers.

Primers (Abgene) were selected on the basis of published sequences of the cDNA of virus genome (Table 1).

Table 1. The primers used for IPNV detection with RT PCR method

PCR	Sequences	Base pairs	Reference
IPN RT	5' CCGCAACTTACTTGAGATCCATTATGC - 3' 5' CGTCTGGTTCAGATTCCACCTGTAGTG - 3'	206 bp	(10)

2.5. RT PCR thermal cycler program.

Reverse transcription of IPNV RNA and amplification of cDNA were performed in a thermal cycler: 47 °C for 60 min and 94 °C for 5 min followed by 40 amplification cycles of denaturation at 94°C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 90 s, and final extension at 72 °C for 10 min.

2.6. Gel electrophoresis.

PCR products were analysed on a 2% agarose gel by electrophoresis in 120 V for 60 min in TAE buffer (sterile water, Tris, acetic acid and EDTA) loaded with 8 to 10 µL of PCR sample. A PCR marker of 100-bp DNA ladder molecular weight was also loaded and run on each gel. The gels were stained in 1% ethidium bromide and a UV transilluminator (Biorad) was used to visualize the bands, and results were recorded by photography. On each gel, negative controls from uninfected material and positive controls were run as well. Laboratory analyses were performed at the animal health service laboratory in Munich in Germany.

3. Results

Samples were analysed for detection of IPNV. In the Table 2 are presented results from samples in year 2010 and results from 2006-2008, indicating differences of the positive IPNV farms and positive IPNV samples.

In the results from second study samples taken in 43 pools, six pools (14.0%) were IPNV positive, and 4 from 18 sampled fish farms (22.2 %) were IPNV positive.

Comparing results with previous study in years 2006-2009, results indicated higher percentage of IPNV positive pools 14.0 % (6 from 43) while first study was 11.5% (13 from 113). While the number of IPNV positive fish farms was in lower, on first study IPNV positive farms were 53.8% (7 from 13) and in the second study was 22.2% (4 from 18). In both studies all pooled fish positive IPNV were virus carriers with no visible clinical signs of disease.

4. Discussion

Infectious pancreatic necrosis (IPN) is an economically significant viral disease of salmonid fish worldwide [3], therefore good preventive and control measures are required. As with most viral diseases of fish, there is no method of curing the IPN disease, once found on a fish farm. Therefore the best way of dealing with diseases such as IPN should be based on preventative measures.

Table 2. Results from examined pools and fish in 2010 period and 2006- 2009

Period of sampling	**Number of examined pools and fish**			**IPNV positive**			
	Farms	Pools	Fish	Pools	%	Farms	%
Examined pools and fish in 2010 period	18	43	260	6	14.0%	4	22.2%
Examined pools and fish in 2006-2009 period	13	113	467	13	11.5%	7	53.8%

In this study good sanitary practices are applied to prevent this disease including: avoiding contact of the pathogen with the young fish, egg disinfection (with Iodophors), and reduction of stress conditions and adequate separation of generations. Preventing the introduction of a pathogen into a new stock of fish has been accomplished mainly by avoiding contact of possible latent carriers with uninfected young fish on farms in already detected IPNV in study conducted in years 2006-2008. The IPNV negative fish farms from study in 2006-2008 are advised to implement general preventive measures and to avoid possible virus transmission from IPNV positive fish farms.

Based on results from this study the preventive measures and implementation of good sanitary practices are shown to be very important on IPNV prevention between fish farms. This is indicated in comparison of the results from study 2006-2008 showing IPNV positive fish farms in rate of 53.8% and results after preventive measures in 2010 with 22.2% IPNV positive fish farms. During this period of time between first and second sampling were not detected any new IPNV positive fish farm, and at least in four IPNV positive fish farms sampled during period 2006-2008 we have not detected IPNV during the sampling in year 2010.

However the number of IPNV positive samples has increased on farms where IPNV was already present, probably advices are not properly followed or IPNV has already become established. This is shown on results from this study where in period 2006-2008 the IPNV positive samples were 11.5 % and in year 2010 the IPNV positive samples were 14 %.

Therefore, it is highly recommended that fish farmers should apply preventive measures to stop spreading the IPNV in particular in countries where no legal powers insist on certification of freedom from viral diseases and control of movements of fish and fish eggs within country. In Jenicic [11] is recommended that countries where IPNV is already established in trout farming, implementing the legislation where the hatcheries have to be free of this virus and by eliminating the IPNV positive trout brood stock.

Thus, fish farmers as shown in this study can greatly contribute to reduce spread of disease agents within their fish farms. Design and following the sanitation protocols is highly recommended including egg disinfection, disinfection footbaths, disinfection of equipments, proper disposal of dead fish, sanitation measure before and after introducing a new fish stock and preferable use of pathogen-free water. Separation of different generation can contribute on reduction of outbreaks of infection diseases in fish farming, this has applied on this study and also recommended from Ruane et al. [9].

Results from this study indicate that the improvements in fish farming sanitary practices can be applied to reduce occurrence of IPN disease outbreaks in rainbow trout farming.

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