PATHOGENICITY OF VHS, IHN AND IPN VIRUSES FOR PATHOGEN FREE RAINBOW TROUT (ONCORHYNCHUS MYKISS) FRY

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Received for publication March 28, 2006.

Abstract

Pathogen free rainbow trout fry, produced in virus free laboratory environment, were experimentally infected via water with Polish isolates of VHSV and IPNV, and IHNV strain received from the Central Reference Laboratory, Arhus. During the experiments, mortality, clinical symptoms, and pathological lesions were recorded. The data achieved demonstrated that the symptoms, pathological lesions, and the course of VHS, IHN, and IPN differed in some aspects from generally accepted opinions on characteristics of the diseases. Our findings could be applied for the preliminary diagnosis of these diseases in rainbow trout fry. On the basis of cumulative mortality rates it should be concluded that the infection with some Polish IPNV isolates could be fatal to rainbow trout fry and for that reason the disease should be subjected to an eradication programme similarly to VHS and IHN.

Key words: rainbow trout, fry, VHSV, IHNV, IPNV, pathogenicity.

Viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and infectious pancreatic necrosis (IPN) are dangerous salmonid fish diseases. VHS causes a great losses of farmed rainbow trout in Poland, in some years up to 10% of the whole country production of this fish (1-5). According to Smail and Munro (9) VHS is the most serious disease of rainbow trout in European Union member states accounting for estimated annual losses of 40 million pound sterling. Serious losses of trout and pacific salmons are caused by IHN as well. Controversial opinions exist concerning the rate of mortality induced by IPNV infections, which otherwise depends on serotype of this virus.

The information on pathogenesis of VHS, IHN, and IPN is based mainly on the descriptions of spontaneous cases and there are not many investigations on the infection induced experimentally in pathogen free fish. The purpose of this work is to compare the clinical symptoms, gross pathology, incubation periods, and the mortality in pathogen free rainbow trout fry population experimentally infected with the Polish isolates of VHSV and IPNV, and also with the reference strain of IHNV. The information achieved in this experiment would be helpful in the preliminary diagnosis of VHSV, IHN, and IPNV infections in Polish salmonid farms.

There is a special need of information concerning IHN. Except one case of IHN identified in Poland which was immediately eradicated, the virus was not isolated up to now (6). For that reason Polish veterinarians and fish farmers lack the experience in this field though the disease could be introduced at any time to Poland from neighbouring countries.

The studies on the IPN pathogenesis are also justified because the cases of this disease in Poland are numerous and the opinions concerning extent of the fish losses due to IHN in Polish inland salmonid farms are controversial (3-5).

Material and Methods

Eggs and milt were received from the salmonid fish farm free of VHS, IHN, and IPN viruses. Fertilization and fertilized eggs incubation were performed in the fish virus free laboratory environment. The incubators with eggs were supplied with oxygenated well water. The temperature of water during incubation of eggs and fish larvae, also for fish fry rearing, was 14°C±1.

The following virus strains and virus isolates were used for experimental infection of rainbow trout (Oncorhynchus mykiss) fry: VHS-10 isolated from spontaneously infected moribund rainbow trouts originating from one of Polish salmonid farms, reference strain of IHN-32/87 received from the Central Reference Laboratory (CRL), Arhus, IPN 4299/05/W-97, IPN 4675/05/W–1551 and IPN 13275/05/W-354 isolated from carrier rainbow trouts in Poland, and reference IPN,,sp” strain obtained from the CRL.

The infections were performed via water and fry was exposed to each of the virus suspensions for 30
min after which the water flow through the troughs was restored. In the first experiment three groups of 20 two-month-old rainbow trout fry each were exposed to the following virus titers: VHS $8.6 \times 10^6$, IHN $13 \times 10^7$, IPN $5.9 \times 10^6$.

In the second experiment four groups of 45 fry, with remnants of yolk sack, were exposed to the following virus titers: IHN $1.3 \times 10^7$, IPN „sp“ $1.3 \times 10^9$, IPN 4675/05/W – 1551 $1.3 \times 10^9$, IPN 13276/05/W – 354 $1.3 \times 10^9$.

For each of these two experiments two control groups were maintained consisting of 20 fish in the first experiment and 45 fish in the second one. The control groups were subjected to sham infection by exposing them to the sterile virus free cell culture medium. All the fish were fed during the experiment trout pellet feed (50%), frozen cyclops (25%) and frozen white mosquito larvae (25%).

During the experiments, mortality, clinical symptoms, and pathological lesions were recorded and photographs were taken. The routine histological sections were made of the whole fish body (cut transversely) and HE stained. The comparison was performed between the histological sections of healthy and IHN infected fishes. The moribund fish were regularly examined for the presence of viruses used for the infection. For this purpose PCR method combined with the virus isolation in EPC, BF-2 and FHM cell cultures was applied.

## Results

The results of experiments were presented in Figs 1a, 1b, 2, 3, 4, 5, 6, 7, 8a, 8b, 9, 10 and 11. The experiments had shown that the disease incubation periods depended on individual fish and ranged as follows: VHS from 5 to 10 d, IHN from 9 to 27 d, IPN „sp”, IPN 4675/05/W – 1551, and IPN 13276/05/W – 354, from 11 to 29 d, from 14 to 29 d, and from 11 to 29 d, respectively.

The mortality differed depending on the virus strain/isolate. There was 100% mortality after 11 d in the case of VHS and after 28 d in the case of IHN. In IPNV „sp”, IPN 4675/05/W–1551, and IPN 13276/05/W–354 infected groups after 30 d the mortality was 71%, 17.8%, 57.8%, respectively.

Some virus infected fish were dying without any visible symptoms or macroscopic lesions.

The presence of the virus used for the infection was confirmed in symptomless and moribund fish in each group. In the first control group none of the fish died, in the second control group two of 45 fish were found dead but in none of them the virus was found.

In the first experiment, in VHSV infected fish the most common symptoms appeared to be darkening of the skin in 6 fish, gill anaemia in 5 fish, petechiae on the fin base in 3 fish, liver blood vessel dilation in 1 fish (Fig. 6), and spleen enlargement in 1 fish. In IPN 4299/05/w – 97 isolate infected group there was no mortality.

In the second experiment, performed in younger fry, in IHNV (with the remnants of yolk sack) infected group skin darkening in 12 fish was the most often noted symptom. Moreover, exophthalmia was found in 6 fish, gross cranial distension in 3 fish (Figs 7, 8a, 8b), pseudofaeces in 2 fish (Fig. 9), and skin petechiae in 1 fish. In IPNV „sp” infected group skin darkening was also most prevalent was observed in 6 fish. Exophthalmia developed in 4 fish. In the case of IPN 4675/05/W–1551 exophthalmia and darkening of the integument appeared in 4 fish. In the group infected with IPN 13275/05/W–354 isolate darkening of the skin was also the most prevalent symptom in 5 fish, moreover, exophthalmia was observed in 3 fish.

The histological examination of IHNV infected fishes demonstrated the deformation of the encephalicus, vacuolization of the brain tissue and the presence of exudate in the encephalicus cavity (Figs 7, 8a, 8b). The substantial destruction of the kidney tubuli and Maliphian corpuscles were also evident.

**Fig. 1a. Normal gills of rainbow trout fry.**

**Fig. 1b. Gill anaemia in rainbow trout fry infected with VHSV.**
Fig. 2. Blood effusion in rump muscle of rainbow trout fry infected with VHSV.

Fig. 3. Blood effusion in body muscle of rainbow trout fry infected with VHSV.

Fig. 4. Blood vessel dilatation in rainbow trout fry infected with VHS.

Fig. 5. Exophthalmia in rainbow trout fry infected with IHNV.

Fig. 6. Liver blood vessel dilatation in rainbow trout fry infected with IHNV.

Fig. 7. Hydrocephalus in rainbow trout fry infected with IHNV.

Fig. 8a. Histological section of normal brain of rainbow trout fry, HE, x 60.

Fig. 8b. Histological section of hydrocephalus in rainbow trout fry infected with IHNV, HE, x 60.
Discussion

In fish infected with each virus except one nonpathogenic IPN isolate, the darkening of the skin was a predominant or relatively often appearing symptom. In VHSV infected group also gill anaemia and blood vessel destruction quite often were seen. In contrary to the descriptions provided by Smail and Munro (9) in our experiments the nervous symptoms, bloated appearance, and fluid in the abdominal cavity were missing in VHSV infected two-month-old rainbow trout fry.

In the case of IHNV infected fry, the most characteristic was exophthalmia accompanied with gill anaemia and occasionally delicate petechiae in the base of the fins.

In very young rainbow trouts, with yolk sack remnants still present, the most common was exophthalmia. Relatively rarely pseudofaeces were seen. One of the most prominent pathological changes found...
Noga (8) usually occurs on day 12 to 18 and in our opinion concerning peak mortality which according to be minimum 11-14 d. Differences also exist in experiment with Polish IPN isolates this period appears incubation period in IPNV infections is 3-8 d and in our Noga (8) and other authors. According to Noga (8) other two IPN strains/isolates used in our experiments. from CRL seemed to be the more pathogenic than the these days.

We found striking differences in incubation period and in the course of disease between VHSV and IHNV infected fish (Fig. 10). The VHSV infection was acute one. The first fish became ill on the 5th d and died 1 d after. During the next 5 d all the fish died. According to Noga (8) incubation period in VHS is usually 1–2 weeks. In our opinion this period depends on individual fish and in 2-month-old rainbow trout fry, according to the results of our experiments, it may range from 5 to 10 d.

In the fish infected with IHNV the shortest incubation period, i.e. when the first moribund fish was observed after infection, was relatively long and lasted 8 d in younger fry and 9 d in older ones. The last IHNV affected older fry died on the 28th d of observation and younger fry on the 23rd d (Fig. 11). Incubation time in IHNV infection according to Noga is 5 – 14 d and it differs from our results.

In the group of fish infected with pathogenic reference strain IPN,,sp” and in groups infected with two Polish field isolates, darkening of the integument and exophthalmitis were the only symptoms. There was no sudden increase in the mortality of these fry and also many symptoms described by Noga (8) in IPNV infection, such as neurological signs (corkscrew, spiral swimming, whirling) and cohesive foecal pseudo cast were missing, except in one fish.

The IPN isolate 4299/05/W–97 proved to be non pathogenic for 2-month-old rainbow trout fry and no mortality or morbidity was observed in the course of 30 d observations.

The pathogenicity of Polish IPN strain 4675/05/W–1551 was relatively low; daily mortality never exceeded 1 fish, cumulative mortality was 17.8% and incubation period 14 d (Fig. 11). Another Polish strain IPN 13276/05/W–354 caused peak mortality on the 22nd and 24th d post infection and 6 fish died during these days.

This strain and the strain IPN,,sp” received from CRL seemed to be the more pathogenic than the other two IPN strains/isolates used in our experiments.

The incubation period in IPN infection in our experiments differs distinctly from the data presented by Noga (8) and other authors. According to Noga (8) incubation period in IPNV infections is 3-8 d and in our experiment with Polish IPN isolates this period appears to be minimum 11-14 d. Differences also exist in opinion concerning peak mortality which according to Noga (8) usually occurs on day 12 to 18 and in our experiments it is evident that it was observed on days 22-24. It seems that the isolates of IPN used in our experiment were less pathogenic than the isolates described by Noga (8). It is also possible that fish used in our experiment were more robust and immune to infection than his fish, and it could be due to good feed quality they were getting i.e. 50% of natural feed was served each day. The results of the performed experiments concerning the symptoms and gross lesions in rainbow trout fry infected with VHS, IHN, and IPN viruses will be very useful for the breeders supervising their hatcheries and veterinarians during recommended inspections according to EU Directive (10). When above characteristic symptoms of moribund fishes (described as a result of experimental infection) appear in the farm, the presence of VHS, IHN or IPN can be suspected and fishes should be immediately sent to the laboratory for virological examination.

It is well understood that realization of control programmes with a purpose of VHS and IHN eradication should be given priority. The IPN virus should be considered as the next for elimination. Our experiment has proved that the mortality in some IPN cases is quite large though the disease could be often chronic and daily mortality low. Cumulative mortality in the case of IPN,,sp” IPN 4675/05/W-1551 and IPN 13276/05/W-354 virus infections were 71%, 17%, 57.8%, respectively.

The probability exists that such evident differences in the pathogenicity of particular IPN isolates are connected with different genotypes. This subject will be studied by us in the near future.

The application of virus free young naïve fish fry produced in laboratory proved to be very valuable for virus pathogenicity assessment and the authors intend to improve and eventually standardize this method for future practical applications.

References


