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FIRST DETECTION OF HIRAME RHABDOVIRUS (HIRRV) IN EUROPE

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Introduction

Hirame rhabdovirus (HIRRV) is one of the four recognized species within the *Novirhabdovirus* genus, represented by the type species *Infectious Haematopoietic Necrosis* (IHNV). HIRRV was first isolated during an outbreak on cultured flounder (*Paralichthys olivaceus*) and ayu (*Plecoglossus altivelis*) in Japan (1). It was also found on other marine fish in Asia, such as stone flounder (*Kareius bicoloratus*) in China (2). Furthermore, it was shown to be pathogenic for a range of salmonids species, including rainbow trout, experimentally challenged in freshwater. The major clinical signs of HIRRV infection were congestion of the gonads, focal hemorrhages of the skeletal muscle and fins and ascitic fluid collection (3).

We report the first description of HIRRV in Europe, isolated from grayling and brown trout in a farm in Poland.

Materials & methods

Thirty adult graylings (*Thymallus thymallus*) with clinical signs and thirty asymptomatic adult brown trouts (*Salmo trutta m. fario*) from the same farm in Poland were tested for the presence of novirhabdoviruses by cell culture. Pools of kidney and spleen from a maximum of 10 fish were homogenized. For virus propagation, epithelioma papulosum cyprini (EPC), fathead minnow (FHM), rainbow trout gonad (RTG) and bluegill fry (BF-2) cell lines were inoculated and incubated at 15°C. Cell cultures were collected for virus identification when cytopathic effect (CPE) appeared, usually 4 to 7 d later.

Starting from RNA extracted from cell culture supernatant, a random-priming sequence-independent single primer amplification (SISPA) was adopted to search for viral sequences (4). PCR products were cloned and sequenced according to the Sanger method.

Transmission studies were carried out on rainbow trout (*Oncorhynchus mykiss*) fry and grayling fry. Virus was propagated in EPC cells and a harvested at maximum CPE, about 4-5 days post inoculation (dpi). Experimental fish were kept in 10 l aquaria supplied with freshwater, the temperature was maintained at 10 or 12°C.

Results

After inoculation on various cell lines, the homogenates from graylings induced a strong cytopathic effect (CPE) after 72 hours, suggesting the presence of a virus.

The virus isolated in cell culture induced mortalities on experimentally infected graylings, reaching 10-25% after 21 days. In moribund graylings, light petechiae and congestions in rump muscles and also in internal organs were observed.

Although some antigenic similarities with perch rhabdovirus (PRhV) were observed, RT-PCR with several sets of generic primers amplifying all fish vesiculo-like viruses, gave consistently negative results. Therefore, we used a sequence independent single primer amplification (SISPA) strategy to obtain and identify viral genomic fragments with similarities to other viruses in GenBank. Surprisingly, of the 60 clones sequenced, two of them showed high sequence similarities (> 99%) with either the L gene or the N gene of HIRRV, a viral species that had been reported only in Japan,

China and Korea till now. By amplifying specifically the P gene, we observed that the virus exhibited a higher identity with the Chinese strain compared to the Korean, suggesting that the virus was imported from China, maybe in frozen food.

A specific qPCR was developed and used to demonstrate that the same virus was also present in cell culture inoculated with brown trouts extracts from the same farm.

Discussion & conclusions

This study identified for the first time the presence of HIRRV in grayling and brown trouts in a farm in fresh water in Europe. The European isolate was highly similar to two other Asiatic strains, from Korean and China. Meanwhile, the sequence of the P gene revealed a stronger similarity with the Chinese strain, which would be consistent with the hypothesis of the introduction of the virus via frozen food imported from China and used in the farm. This finding raises concerns about the spread of this virus out of Asiatic countries and its potential emergence in freshwater conditions.

Any symptom was visible on the graylings and brown trouts from the affected farm, suggesting a latent infection. However, the virus, once produced in cell culture, provoked mortalities during an infectious challenge on graylings and rainbow trouts. The conditions of virulence should be further investigated to estimate the epizootic risks in Europe on grayling and other freshwater fish. It must be mentioned that at the same period of viral isolation, a massive mortality occurred on wild grayling in a river in the same region. Although no samples could be analyzed at that time, the possibility of an HIRRV outbreak is hypothesized. We now have the specific diagnostic tools for routine surveillance and investigationof any other mortality event.

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