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VHSV CAME FROM THE MARINE ENVIRONMENT: CLUES FROM THE LITERATURE, OR JUST RED HERRINGS?

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Abstract

Viral haemorrhagic septicaemia (VHS) was for many years found only in freshwater fish, primarily the rainbow trout (*Oncorhynchus mykiss*), but the causative virus (VHSV) has now been isolated from an increasing number of marine fish species. The marine fish may have been infected by virus from infected freshwater fish passing from rivers into the sea, but there is also the possibility that marine fish were the initial hosts of the virus, and it was passed from a marine reservoir to the freshwater environment by the feeding of marine fish to rainbow trout. Much of the latter hypothesis is speculation. However, the early literature on the disease provides firmer ground to support that hypothesis; that literature is reviewed.

In recent years there has been an increasing number of reports of the isolation of viral haemorrhagic septicaemia virus (VHSV) from fish from the marine environment (Meier *et al.*, 1994, Ross *et al.* 1994, Smail 1995, Meyers and Winton 1995). Such reports have been accompanied by speculation as to the origin of the marine VHSV; in particular, whether the virus originated in freshwater and was subsequently transferred to the marine environment, or *vice versa.* VHSV was isolated recently at this laboratory from Atlantic herring (*Clupea harengus*) from the English Channel (Dixon *et al.*, 1997). In that paper we speculated that feeding herring to rainbow trout (*Oncorhynchus mykiss*) cultured in freshwater may have facilitated the transfer of VHSV from the marine to the freshwater environment. A re-reading of early works on the disease now known as VHS provides further pointers in that direction; there are also other indications from the literature that, although not realised at the time, VHSV from marine sources may have been transferred to both rainbow trout and other species cultured in the marine environment. That literature is reviewed here.

It is purely conjectural as to whether all of the early reports of VHS disease, prior to the use of cell cultures for diagnosis, were actually caused by VHSV. One of the conditions later accepted (Anon 1963) as being the same as VHS disease was "Nierenschwellung" (kidney swelling) (Schäperclaus 1938), later renamed "Infektiöse Nierenschwellung und Leberdegeneration" (INuL) (infectious kidney swelling and hepatic degeneration) (Schäperclaus, 1954), but this condition actually may have had another cause, such as proliferative kidney disease (PKD), or a combination of VHSV and PKD, as discussed by Clifton-Hadley *et al.* (1984). Certainly in many of the cases of INuL there were none of the clinical signs now associated with VHS disease, and it should be noted that Nierenschwellung was originally described as a disease occurring at warm water temperatures, in summer (Schäperclaus, 1938), which is not typical of VHS. The kidney swelling was later described as being a "rather uncommon" sign of the disease (Ghittino 1973). Hence probably not all documented cases of Nierenschwellung or INuL were actually VHS. However, in his later description of the condition, Schäperclaus (1954) described its occurrence at a trout breeding station at water temperatures of 2-6°C. About 70% of the fish had swollen kidneys and the remainder had signs which could possibly be those of VHS. Fish from that site were used for the filtration and transmission experiments which lead to the conclusion that a virus was involved in the disease, and therefore it is known that VHSV was present in at least some of the fish. The significant feature of that report was that the author also noted that the trout had been fed on marine fish. Interestingly, Schäperclaus (1954) also related

that in 1952 and 1953 the disease was becoming widespread in Europe, and that the disease pattern was changing - it was now mainly occurring at cold water temperatures (and with more typical signs of VHS, including haemorrhages); perhaps that was the start of the "real" VHS epizootic. Schäperclaus was convinced that the intensive feeding of fresh herring played an important part in causing some of the signs associated with the disease. If the supposition of Dixon *et al.* (1997) that the herring is a reservoir of VHSV is correct, some of those herring could have been infected with VHSV virus, and hence the virus could then have passed into the rainbow trout.

It was in the early 1950s that the disease was first recognised in Denmark (by Prof. Schäperclaus) (Christensen *et al.* 1963). Christensen (1965) related that rainbow trout cultivated in Denmark were fed on a diet of marine fish such as herring, mackerel (*Scomber scombrus*) and sand eels (*Ammodytes* spp.) which were transported daily from fishing ports to the fish farms. Marine fish as a food source for rainbow trout were implicated in several of the early theories as to the cause of the disease now known as VHS, although those theories were based on the poor nutritional value of some of the marine fish. Lauridsen (1958) (cited by Rasmussen 1965) suggested that the disease was caused by a lack of vitamins B_1 and E from feeding herring to rainbow trout. Besse (1958) also suggested a nutritional cause for the disease, but in that case, the cause was related to the feeding of marine fish that were not fresh. Significantly, one of the factors put forward to support this theory was the fact that, in France, the disease was a hatchery disease, and that most of the hatcheries affected were near the sea. At those hatcheries practically all the food was marine fish. If marine fish were the source of VHSV, it would be expected that predominantly sites near the sea would be infected, as marine fish as a food source were not used to the same extent further inland.

In the cases related above, the feeding of marine fish, particularly herring, has been noted by the early investigators, either just as a record of fact, or as being of significance to the disease. The movement of infected fish or possibly, but less likely, infected eggs would subsequently have transferred the disease to inland sites. Such a cause was suggested by Håstein *et al.* (1968) for the appearance of VHSV in Norway. Several possible VHSV outbreaks were reported, although according to that reference only one of them was confirmed virologically. In that outbreak, fry, one- and two-year old fish were affected, and included fish reared in both fresh water and, significantly, in sea water. The authors reported that it was difficult to determine the source of the infection. Fish were not imported onto that site, which was in an isolated location, and the spread of infection from other sites was ruled out. However, eyed eggs had been introduced onto the site; the source of the eggs was not recorded, and so they may have been from a VHSV-positive establishment. Even if that were so, VHSV was unlikely to have been present on eyed eggs (Jørgensen 1970, Ghittino 1973) although virus transmission by eyed eggs has been recorded (Ghittino 1965). In this case in Norway, could there actually have been a marine source of the virus, leading to this early report of VHSV in rainbow trout reared in sea water?

Castric and Kinkelin (1980) also reported the occurrence of a natural outbreak of VHSV in rainbow trout reared in a cage in sea water in France. The fish had been stocked from a VHS-free farm, and the source of the infection was ascribed to fish in a nearby cage, some of which had come from Normandy. As VHS was present in Normandy at the time, the easiest explanation for the VHS outbreak was that those fish had brought in the virus, but no data were presented to show that the actual source of the fish in Normandy was indeed VHSV-positive. Furthermore, one curious feature of this report was that the VHSV outbreak in the fish stocked from the Normandy site started *after* the outbreak in the cage stocked with fish from the VHSV-free site. If the fish from Normandy were infected before being put

into the sea cages, and transmitted the virus to the other cage, it would seem most likely that they would show signs *before* the other fish. An alternative explanation is that the fish from the disease-free site were actually infected with VHSV from a marine fish; the virus may then have spread to the fish in the second cage (fish from Normandy) or they too may have been directly infected from a marine fish.

Instances of VHSV occurring in rainbow trout reared in Danish coastal areas were recorded by Hørlyck *et al.*, 1984. They reported outbreaks of VHSV in several marine sites, and although the source of infection in some of these cases may have been from incompletely disinfected vehicles, the authors reported that they thought the source of infection to be "obscure". A possible explanation in one case was that the virus had been transmitted from another site 2 km away, which had been stocked with VHSV infected rainbow trout. However, the authors thought that because of the massive dilution effect in sea water, such an explanation was unlikely. The authors had considered a virus reservoir in the marine environment, but did not discuss that possibility further as at that time there was no evidence for such a reservoir. That explanation now seems most likely. The same conclusion was reached by Jørgensen (1992) when considering further instances of VHSV infections in maricultured rainbow trout in Denmark.

A further suggestion that rainbow trout may have been infected with a virus from the marine environment comes from a report by Ahne *et al.* (1986). They reported that two VHSV isolates from trout reared in brackish water in Germany were not neutralised in standard tests, although they did react in the indirect immunofluorescent antibody technique. Those isolates were obviously different from the usual isolates found in Germany at that time. The previous history of the fish was not reported, but if they were transferred to the site and were already infected with VHSV, it would be expected that they would be infected with one of the usual German virus types. As that was not the case, the fish may have been infected with the unusual virus at the brackish water site, and the marine environment is a possible source of the virus. To counterbalance that argument, however, are reports that serological variants of VHSV, which apparently have no connection with the marine environment, have been isolated (Olesen *et al.* 1993, Fichtner *et al.* 1998).

The above examples are of maricultured rainbow trout possibly being infected with VHSV from the marine environment. The authors reporting them mostly discussed the cases in relation to a freshwater source of the virus, which was understandable given the knowledge (and dogma) at the time. However, with current knowledge, a marine source of the virus is just as, or more, likely.

Natural outbreaks of VHSV have occurred in turbot (*Scophthalmus maximus*) cultured in German, Scottish and Irish marine waters (Schlotfeldt *et al.*, 1991, Munro, 1996, McArdle, personal communication 1997). In the latter two outbreaks the origin of the virus was not determined for certain, but in both cases the original sources of the fish were VHSV-free, and both countries were otherwise VHSV free. The only realistic source was from the marine environment, either from a food source, or direct transmission from infected marine fish. All three VHSV isolates from the turbot shared a high degree of sequence homology with VHSV isolated from cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) (Smail, 1995, Smail cited by Munro, 1996) (Stone *et al.*, 1997, Stone, personal communication 1998). The source of the virus for the turbot in Germany was thought to be from contaminated water. The authors reported that there were trout rearing units 25 to 35 km away, but also stated that they did not know whether those units were VHSV-positive or not. Even if they were positive, the chances of infectious doses of virus travelling 25 to 35 km must be extremely remote, considering the dilution factor, and so a marine reservoir is a more likely source of the virus. It is interesting to note that this turbot rearing

site was on the Baltic coast of Germany. Geographically, this is near to the catch site of the cod from which the first marine VHSV was isolated (Jensen *et al.* (1979).

There is therefore this circumstantial evidence that VHSV may have been transmitted from marine fish to rainbow trout and cultivated marine species. However, one problem with this theory is the finding that under experimental conditions, VHSV isolates of marine origin are completely avirulent (Winton *et al.* 1991, Jørgensen 1992, Dixon *et al.* 1997, Avery and Dixon unpublished data) or have low virulence (Meyers *et al.* 1994) for rainbow trout. How, then could VHSV have been transmitted from the marine to the freshwater environment? Several mechanisms are possible, including the following:

a) over a number of years of continuous feeding of virus-infected food, eventually, by chance, one or more mutant viruses from the quasi-species in any virus population had an increased virulence for the rainbow trout.

b) the rainbow trout were more susceptible when stressed and then fed virus-infected food. Certainly it is stressful for rainbow trout to be transferred to seawater and hence they may then be more susceptible to the virus from a marine source. There are also many sources of stress for fish in freshwater e.g. overcrowding, other infections, poor water quality, poor nutrition, any of which may predispose the trout to VHSV infection. The latter factor may have been caused by vitamin deficiency through feeding marine fish, or the marine fish may not have been fresh. Paradoxically, those factors were proposed as the direct cause of the disease by Lauridsen (1958) and Besse (1958) as described earlier in this review!

Transport of live fish is also stressful, and there are several reports of the disease occurring in fish which have been transported from one region or country to another (Anon. 1972, Schäperclaus 1954, Ghittino 1973).

c) The feeding of marine fish, particularly from the Baltic Sea, may have immunosuppressed the rainbow trout, and rendered them more susceptible to the virus. The Baltic Sea contains contaminants, including polyhalogenated aromatic hydrocarbons (PHAH), which are also found in the food chain. It has been shown that feeding contaminated herring from the Baltic Sea to seals resulted in impaired immune responses (see Ross *et al.* 1996), and feeding such fish to rats resulted in higher titres of rat ctyomegalovirus in salivary glands after challenge with the virus, compared to rats fed herring from the Atlantic (Ross *et al.* 1996). It is interesting to note that the Baltic Sea area has been a long-term source of the virus. The first marine fish (cod) from which VHSV was isolated were caught there (Jensen *et al.* 1979), a mariculture facility in which there was a natural outbreak in turbot was located there (Schlotfeldt *et al.* 1991), the disease in Denmark first occurred in eastern Jutland (Rasmussen 1965) which faces the Baltic Sea, and recently there have been many isolations of VHSV from several marine fish species from the Baltic Sea (Heuer *et al.* 1997). The Baltic Sea could also have been a source of marine fish for feeding to rainbow trout in Germany, which is where some of the early instances of the disease occurred. Hence the feeding of PHAH-contaminated herring from the Baltic Sea to rainbow trout in fresh water may have immunosuppressed the fish, rendering them more susceptible to VHSV which was also present in the herring.

All of this is supposition, but I hope it is a reasonable interpretation of the early literature. It does appear that there is evidence for VHSV originating in the marine environment and being transferred to freshwater, and not *vice versa*. Although not all the examples I have cited will be generally accepted as supporting this thesis, I believe that most of the red herrings in this review are haemorrhaged ones! There have been recent reports that Baltic herring are being appraised as a feed for maricultured rainbow trout (Ruohonen, 1998a,b); I await with interest reports on the health status of those fish.

Note:
A "red herring" is an English phrase meaning a false clue

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