First occurrence of viral haemorrhagic septicaemia virus in rainbow trout in the United Kingdom: studies on the virulence of the virus for rainbow trout and brown trout

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Introduction

Viral haemorrhagic septicaemia (VHS) is a serious rhabdovirus disease of rainbow trout, *Oncorhynchus mykiss*, and other species, and is listed as a notifiable disease by the World Organisation for Animal Health (Office International des Epizooties, OIE) and is categorised as a List II disease (serious endemic disease) by the European Union. The causative virus, VHS virus (VHSV), has been identified in freshwater fish, particularly salmonids, in Europe, North America, several Asian countries including Japan and has also been identified in many marine fish species in the same broad geographic areas (Essbauer and Ahne, 2001; Skall *et al.*, 2005). The United Kingdom (UK) mainland is free from VHS, although in 1994 a marine isolate of the virus was isolated from turbot, *Scophthalmus maximus*, reared in tanks on the island of Ghia, Scotland (Ross *et al.*, 1994). Following successful eradication of the virus from the site and subsequent monitoring for the virus, the VHS-free approved-zone status of the whole of the UK was re-established.

However, in May 2006, VHSV was isolated from a rainbow trout farm in Yorkshire, England. The trout had been undergoing chronic mortalities for several weeks previously, and initially the bacterial disease enteric redmouth had been suspected to be the cause of death by a health specialist employed by the farm. However, following failure of the trout to respond to antibiotics, a comprehensive investigation by Cefas resulted in the isolation of VHSV. As part of a wider epidemiological investigation into the circumstances and consequences of the disease occurrence, experiments were undertaken to determine the virulence of the virus for rainbow trout, particularly as only chronic mortality had been observed in that species on the farm. The virulence of the virus for brown trout, *Salmo trutta*, was also investigated as that was the predominant susceptible species in the river into which infected water from the fish farm was discharged.

Materials and methods

The VHSV isolate (J167) from the fish farm in Yorkshire and a reference isolate from Denmark (3592-B, provided by Dr N Lorenzen, National Veterinary Institute, Århus), were grown and titrated in BF-2 cells at 15°C. Rainbow trout with a known health status were obtained from four different sites as previous studies had shown that some stocks of fish were more resistant to VHSV than others (S Baynes and R Paley, this laboratory, unpublished data). The average weight of the different groups of fish ranged from 4.6 to 15.8 g. The fish from the different sites were mixed in tanks containing 40 l water at 10°C, such that each tank contained 120 fish. For challenge, the water flow to the tanks was stopped and virus was added to duplicate tanks; duplicate control tanks received an equivalent volume of maintenance medium. The water flow was resumed after 4 h. For infection of rainbow trout J167 was added at concentrations of 10^1, 10^2, 10^3, 10^4 and 10^5 $TCID_{50}$ ml^{-1},and 3592-B was added at 10^5 $TCID_{50}$ ml^{-1}. A similar procedure was used for infecting duplicate tanks of brown trout, 50 fish per tank (average weight 5 g). They were infected with J167 at concentrations of 10^1, 10^3 and 10^5 $TCID_{50}$ ml^{-1}. As a positive control, rainbow trout from a single source (average weight 0.5 g) were infected with J167 at 10^5 $TCID_{50}$ ml^{-1}. When the cumulative mortality in a tank exceeded 80% and the mortality rate appeared to be slowing, the remainder of the fish in the tank were humanely killed.

Acknowledgement

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Results

The VHSV challenge of rainbow trout is shown in Figure 1 and that of brown trout is shown in Figure 2. Mortality was confirmed as being caused by VHSV by re-isolation of the virus in cell culture and identification by reverse transcription polymerase chain reaction. The most obvious clinical signs observed in rainbow trout were darkening of the fish, exophthalmia, with or without periocular haemorrhages (Figures 3 and 4) and pale gills, with or without haemorrhages (Figure 4). Internally the liver was pale, but consistently the spleen was dark. The kidney was often dark, but with pale patches. Approximately 50% of the fish had haemorrhages in the muscle (Figure 5).

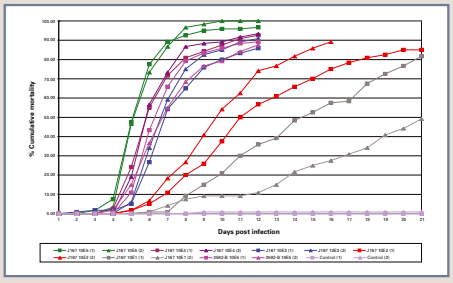

Figure 1: Rainbow trout challenged with different concentrations of VHSV J167, with positive control VHSV 3592-B. Duplicate tanks of fish were infected.

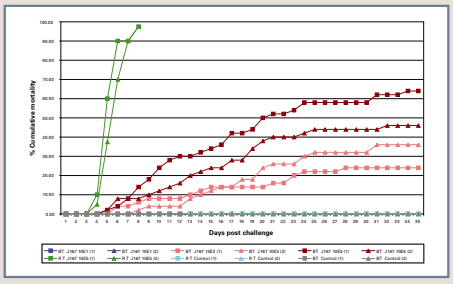

Figure 2: Brown trout challenged with different concentrations of VHSV J167. Rainbow trout challenged with VHSV J167 acted as positive controls. Duplicate tanks of fish were infected. BT, brown trout; RT, rainbow trout.

Figure 3: Rainbow trout infected with VHSV J167. Live fish with bilateral exophthalmia, and darker colour compared with the other fish.

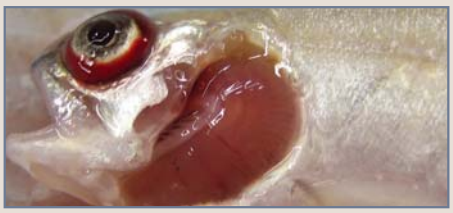

Figure 4: Rainbow trout infected with VHSV J167 exhibiting exophthalmia with periocular haemorrhages. Note the pale gills with haemorrhages.

Figure 5: Rainbow trout infected with VHSV J167 exhibiting haemorrhages in the dorsal musculature.

Discussion

The UK VHSV isolate J167 was clearly highly virulent for rainbow trout fry at all concentrations tested, producing cumulative mortalities of 80-100% by 12 days post infection. The mortality in the replicate tanks for each virus concentration was very similar at concentrations of 10^3 - 10^5 $TCID_{50}$ ml^{-1}, whereas at 10^1 and 10^2 $TCID_{50}$ ml^{-1} there was greater variation between replicate tanks, particularly at the lowest virus concentration tested. At the higher virus concentrations the majority of the fish are likely to have received a lethal dose of virus shortly after the start of the challenge, which resulted in a rapid infection and high mortality. Conversely, at the lower virus concentrations, not all fish may have received a lethal dose at an early stage, and only became lethally infected following production of virus by other fish in the tank that had received a lethal dose. Hence the virus levels in those duplicate tanks may have varied widely after the initial infection ,giving rise to the observed differences in the mortality rate and the cumulative mortality.

The brown trout were much less susceptible to the virus, reaching an average of 55% mortality by 35 days post infection with a virus concentration of 10^5 $TCID_{50}$ ml^{-1}. Interestingly, the brown trout mortalities started shortly after the first mortalities were seen in the positive control rainbow trout, but the progression of the disease was far slower. The difference in susceptibility of rainbow trout and brown trout is in accordance with previous observations. One VHSV isolate from rainbow trout, at a concentration of 10^5 plaque forming units ml^{-1}, produced 32% mortality in brown trout (average weight 6 g), compared with 58% mortality in rainbow trout (average weight 5 g) (Jørgensen, 1980), but five other isolates from rainbow trout did not cause any mortality in brown trout. In the same study, two VHSV isolates from brown trout were pathogenic for both brown trout and rainbow trout. However, Glass *et al.* (1991) challenged brown trout with four VHSV isolates and recorded insignificant mortalities (0-1.5%) compared with mortalities ranging from 73-100% in rainbow trout.

In summary, VHSV isolate J167 poses a significant risk to both juvenile rainbow trout and juvenile brown trout.

References

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