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Genetic diversity and phylogenetic classification of viral hemorrhagic septicemia virus (VHSV)

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Summary — The present study was undertaken to determine the genetic diversity of viral hemorrhagic septicemia virus (VHSV) and to gain insight into the molecular epidemiology of this fish rhabdovirus. The sequences of the nonstructural (NV) protein and the transmembrane (G) protein of sequential North American and European isolates of VHSV were determined and used to compute phylogenetic trees. According to the percentage of nucleotide or amino acid similarities, North American and European isolates formed 2 clearly distant genetic groups. While North American isolates clustered into a highly homogeneous genetic group, European isolates exhibited a higher genetic variability. Sub-grouping based on this variability could be correlated with both the geographic origin and the serological classification.

VHSV / glycoprotein / NV protein / sequence / genetic diversity / phylogeny / rhabdovirus

Résumé — Diversité génétique et classification phylogénique du virus de la septicémie hémorragique virale (VSHV). Le présent travail a été entrepris dans le but de déterminer la diversité génétique du virus de la septicémie hémorragique virale (VHSV) et réaliser une étude d’épidémiologie moléculaire. Les séquences des gènes de la protéine non structurale (NV) et de la glycoprotéine transmembranaire (G), d’isolats séquentiels du VHSV, provenant d’Europe ou des États-Unis, ont été déterminées et utilisées pour calculer des arbres phylogénétiques. Sur la base du pourcentage de similitude des séquences, les souches américaines et européennes se classent en 2 groupes génétiques clairement séparés. Alors que les souches américaines forment un groupe extrêmement homogène, les souches européennes sont génétiquement plus variables. Cette variabilité permet de les regrouper en sous-groupes génétiques qui sont corrélés à la fois à l’origine géographique et à la classification sérologique.

virus de la septicémie hémorragique virale (VSHV) / glycoprotéine / protéine NV / séquence / diversité génétique / phylogénie / rhabdovirus

* Correspondence and reprints
INTRODUCTION

Viral hemorrhagic septicemia virus (VHSV) is a fish rhabdovirus that produces severe losses in the European trout farming industry (de Kinkelin et al., 1979). The virus usually causes a systemic disease among juvenile rainbow trout, *Oncorhynchus mykiss*, with mortality rates as high as 90%. An extensive enzootic virus exists in occidental Europe, where fish farms showing regular appearances of the disease are known. VHSV was isolated in western North America (Hopper, 1989), from adult coho salmon, *O. kisutch* during a routine control (Brunson et al., 1989). Serological analyses using polyclonal antisera were not able to distinguish between the American isolates and the European reference strains, 07-71. *In vivo* investigations in trout and salmon showed that the isolates from North America were significantly less virulent (Winton et al., 1991).

VHSV belongs to the *Rhabdoviridae* family. It is composed of 6 viral proteins: the nucleocapsid protein (N); a polymerase-associated protein (P or M1); a matrix protein (M or M2); a glycoprotein (G); an RNA polymerase (L); and a non-structural protein (NV). The virus contains a nonsegmented RNA molecule of approximately 12,000 nucleotides (nt) with the following genome organization: 3'-N-M1-M2-G-NV-L-5'. The protein G is responsible for the production of neutralizing antibodies. Four serotypes based on neutralization tests have been described.

Improving our knowledge of the genetic relationship among the diverse isolates of VHSV may lead to a better understanding of the epidemiology and biology of this fish rhabdovirus. Molecular epidemiology studies are based on the comparison of viral isolates at the genomic level. This information may be obtained by different techniques, such as RFLP, RNA fingerprinting or sequencing. Recently, Oshima et al. (1993) compared 8 isolates from North America and Europe by T1 ribonuclease fingerprinting analysis. Their results indicated that the isolates from different continents could be placed into 2 different fingerprint groups. Sequencing has been shown to be a more accurate method of providing approximations of the overall genetic relationships among RNA virus isolates. At present, all the VHSV genes, except the L gene, have been sequenced for the 07-71 and Makah strains (Bernard et al., 1990; Thiry et al., 1991; Bernard et al., 1992; Benmansour et al., 1994).

A genetic study of the VHSV isolates from various outbreaks and stages of epizootic in Europe as well as isolates from North America was undertaken to clarify the epidemiology of VHSV. To surpass the limitations of conventional cloning, we used PCR amplified products as sequencing templates.

RESULTS AND DISCUSSION

In this study, we used 17 VHSV isolates. The 4 serotypes of VHSV are represented. They were isolated in Europe and North America from different fish species. Some of them are sequential isolates from the same fish farm. We selected to study the glycoprotein gene as it codes for the only surface protein. We expected that the variability for this gene would correlate with the serological variability. We also selected the NV gene as it codes for a nonstructural protein of unknown function and we expected that the variability for this gene would be representative of the variability due to random mutations.

The glycoprotein

Sequence comparison

The sequence determination of the glycoprotein gene for the strains of VHSV representative of different serotypes and geo-
graphic locations, allowed a sequence comparison with the glycoprotein of other rhabdoviruses. Only the IHNV glycoprotein was significantly related to VHSV. However, a block of 60 amino acids was identified as having a significant sequence similarity with rabies virus glycoprotein. This block probably corresponds to a region of the lyssavirus glycoprotein where a common structural feature or biological function resides.

Genetic heterogeneity

Comparison between the different serotypes showed that the glycoprotein gene of VHSV is highly conserved, with only 1 to 3% divergence at the nucleotide level and 2.2 to 4.7% divergence at the amino acid level. However, Makah strain, the reference strain for the American isolates, which is serologically related to serotype 1, has 13 to 14% divergence at the nucleotide level and 7.3 to 8.3% divergence at the amino acid level with the European isolates. This low genetic variability was further demonstrated by the fact that we found only a 6 nucleotide difference between 2 isolates of VHSV taken at a 16 year interval from the same chronically infected fish farm. Thus, the evolution rate of VHSV in the field could be roughly estimated at 0.35 nt/year.

Quasispecies

Such a high genetic stability is not usual for rhabdoviruses. In the case of VHSV it did not result from a higher fidelity of the RNA polymerase. The sequence heterogeneity (quasispecies nature) of a given viral population was in the range of those described for rabies virus (Benmansour et al, 1992), and we recorded mutation frequencies under a selective pressure of $4 \times 10^{-4}$ to $1 \times 10^{-5}$, which are similar to those reported for mammalian rhabdoviruses. Thus, the VHSV genetic stability remains unexplained. It may result from the action of unknown genetic bottlenecks, or from the low efficiency of the fish immune system, or from the more stringent constraints imposed on the glycoprotein structure by the low temperature dependence of VHSV.

Phylogenetic analysis

The sequence of the entire open reading frame of the glycoprotein gene was used to compute phylogenetic trees by 3 different methods (maximum parsimony, neighbor-joining, and UPGMA). Computations were carried out on 100 data sets which were randomly resampled from the original aligned sequences. The 3 methods gave comparable results. Two lineages were clearly segregated. The first was composed of the strains from Europe, and the second the North American strains. According to the estimated evolution rate of 0.35 nt/year, the 2 lineages may have diverged from a common ancestor some 600 years ago.

Within the European lineage, the strains could be grouped into 5 genotypes. Four of them corresponded to the 4 serotypes described classically. The 5th comprised 2 strains which belong to serotype 1, but which had been isolated in Northern Europe instead of France. Thus, the genetic classification based on the G sequence was correlated, first with the geographic origin, and then with the serotype.

The nonstructural protein NV

Sequence comparison

The NV gene codes for a nonstructural protein of an unknown function. A similar gene is also present in the IHNV genome, but it is absent in mammalian rhabdoviral genomes. However, in the rabies virus genome a non-coding sequence of approximately the same size is found at the same position and was postulated to be the remnant of an ancestral rhabdoviral gene (Tordo et al, 1986). The
NV protein is not conserved at all between VHSV and IHNV and there is only 71% conservation between the NV protein of the American Makah strain compared to the European 07-71 strain.

Genetic heterogeneity

In contrast to this high sequence divergence between the 2 lineages, the NV gene is extremely well conserved among 9 different isolates of VHSV from North America and Alaska. We observed complete sequence conservation among 8 isolates, and only one nucleotide substitution in another isolate. This sequence conservation is striking, when the host and geographical differences of the analyzed isolates are taken into consideration. The NV gene is also conserved, but to a much lesser extent among the European isolates. We recorded a maximum divergence of 4.6% in nucleotides and 8.2% in amino acids between serotype 1 and serotype 2, which were the most distant serotypes.

Phylogenetic analysis

Similar to the findings for the glycoprotein gene, the 2 different lineages were clearly separated. The first comprised all the strains from North America, which were grouped into one highly homogeneous cluster. The second comprised strains isolated in Europe, which were grouped into 4 genetic clusters. However, these clusters did not match the serotype classification, except for the strains representative of serotype 2, and serotype 4, which could be separated from the other serotypes. The genetic clustering was not correlated either with the geographic origin or chronology. Thus, a phylogenetic analysis based on the NV gene is probably not sufficiently accurate for a precise molecular epidemiology study.

In conclusion, this phylogenetic analysis demonstrated the existence of 2 clearly different lineages circulating in 2 geographical areas, North America and Europe. Our data suggested that the 2 lineages became separated a long time before fish farming was established on both continents.

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