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SPANISH ISOLATES AND REFERENCE STRAINS OF VIRAL *Haemorrhagic septicaemia* VIRUS SHOW SIMILAR PROTEIN SIZE PATTERNS

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Introduction

Viral haemorrhagic septicaemia produced high mortalities in trout farms in Spain during 1984-1986 as in other European countries (Jimenez et al, 1988 a, b; de Kinkelin, 1972; Lorenzen et al, 1988; Winton et al, 1988).

The aim of the present study was to obtain more information about 5 Spanish VHS isolates (Jimenez et al, 1988 a, b) and to compare them with VHS virus reference strains. We report here identical protein molecular weights among VHS virus strains but some hetereogeneity in the region of the N protein.

MATERIALS AND METHODS

Viruses. The strains of virus used were VHS-F_1 at passage level 255, VHS-F_2, IHNV, all provided by Dr. P.E. Vestergaard-Jørgensen, and VHS-23.75 provided by Dr. P. de Kinkelin. Five VHS isolates (Jimenez et al, 1988 a, b) were obtained from fish tissue samples in Spain. The viruses were isolated from: rainbow trout, *Salmo gairdneri,* Richardson (689, from Galicia in 1984; 471, from Navarra in 1986; and 144, from Salamanca in 1984); *Salmo salar* L. (472, from Cantabria in 1986) and *Barbus graellsi,* Steindachner (798, from Aragón in 1986).

Cells, media and virus purification. Epithelioma papillosum cyprini (EPC) cells were used throughout the experiments. Cell culture technique (EMEM medium, Flow, Ayrshire, Scotland), and virus purification were essentially as reported by de Kinkelin (1972) and modified by Basurco and Coll (1989).

Isotopic Labelling of Virion Proteins. EPC cells monolayers at 90% confluence, cultivated in 96 microwell plates (0.3 ml per well) were infected with 1-5 $TCID_{50}$ per cell with 2% foetal calf serum. After 1 h of adsorption at 14°C, 150 µl of cell culture media with 2% FCS was added to each well. Cells were incubated at 14°C. until 70% cytopathic effect (about 36-48 h). At this time culture medium was removed, and 40 µl of methionine-free medium were added. Fifteen min later, medium was replaced with 40 µl of methionine-free medium containing 200 µlCi ^{35}S-methionine/ml (Amersham, Paris, France), and maintained for 2 h or 3 days at 14°C. The label was removed and the remaining cells were washed twice with 50 µl of serumfree medium, pH 7.4. The cells were then lysed with 40 µl of gel electrophoresis buffer and stored at −70°C. The separation of the SDS dissociated proteins by SDS-polyacrylamide gel electrophoresis (PAGE) was performed according to Coll (1978). The 0.8 mm thick gels were composed of a slab of 15% or 15-20% polyacrylamide gradient with a stacking gel of 6% polyacrylamide. Samples were boiled for 2 min in SDS denaturing electrophoresis buffer. Samples were then electrophesed under constant current conditions at 30 mA. The slab gels were stained with Coomassie blue, dried onto cellophane and exposed to Agfa Curix RP-2 Xray film for 1-4 days at room temperature before development (Basurco and Coll, 1989).

RESULTS AND DISCUSSION

Our five VHS isolates were neutralized using anti-VHS F_1 antiserum (titre 1

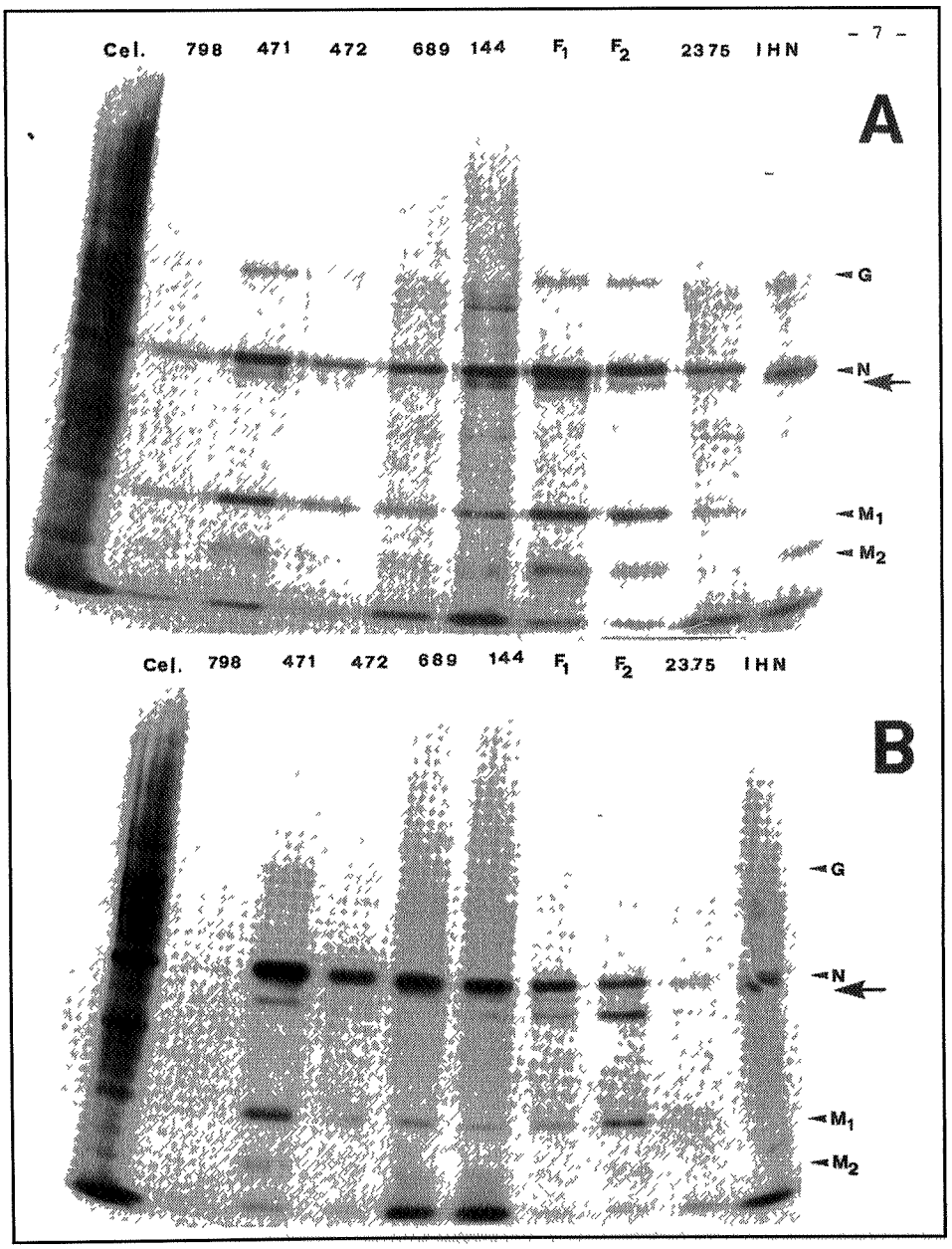

Figure 1. Autoradiography of ^{35}S-methionine labeled cells after infection with different VHS isolates.
Pulses of ^{35}S-metionine were given 40 hours after infection at m.o.i. 1-5 $TCID_{50}$/cell during 2 h (A) or 3 days (B). G, N, M_1,M_2 are the viral proteins of VHS as identified by molecular weight markers run in parallel and as described by Kinkelin et al (1980) and Lorenzen et al (1988).

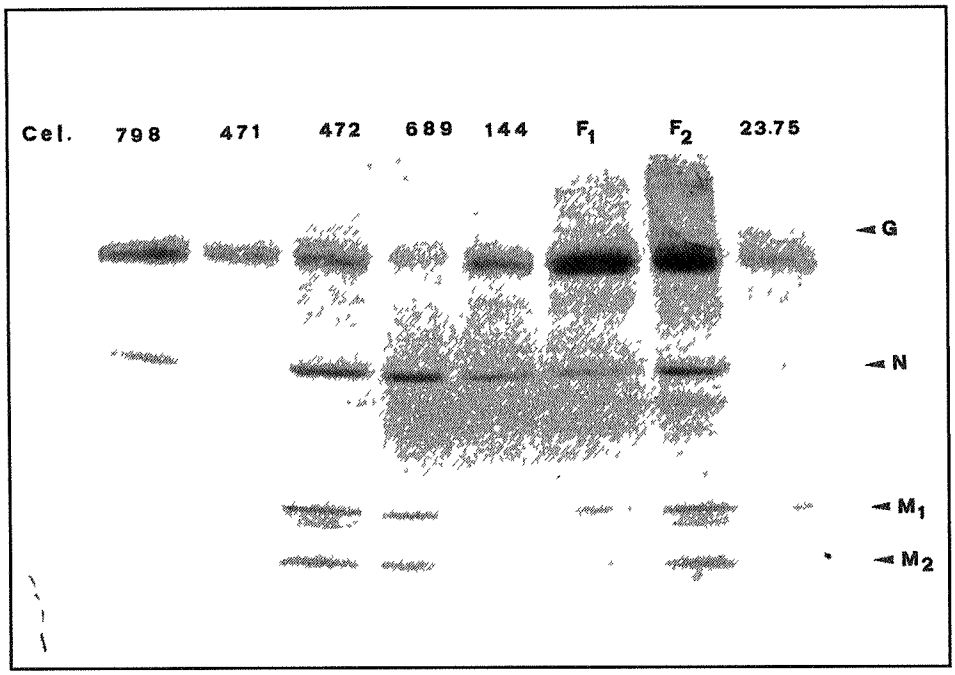

Figure 2. Coomassie-blue PAGE patterns of isolated virions purified from different VHS isolates. Viral infections were allowed to go until complete cytopathic effect and then supernatants were PEG precipitated, ultracentrifugated and concentrated by lyophilization prior to SDS—PAGE.

: 500) given by P.E.V. Jørgensen (Jimenez et al, 1988 a, b; Basurco and Coll, 1989). Two different methods of detecting viral proteins were used by SDS-PAGE: either purified virus, or infected cell extracts labelled with ^{35}S-methionine. For isotopic labelling a 70% cytopathic effect of the infected monolayer was chosen as the moment for labelling, because the background of host cellular synthesis is minimal at this time. With this labelling time, harvest of remaining cells was either 2 h later or 3 days later.

The first noteworthy observation was that no differences in molecular weight of each of the proteins was found among the Spanish isolates and the standard viruses by any of the methods employed. The proteins detected were the L (200 KDa), G (65 KDa), N (33-38KDa), M_1 (24 KDa) and M_2 (20 KDa). The L protein was not detected in all experiments or in some isolates, but this could be due to its low concentration as reported by others (Lorenzen et al, 1988). Some small variations in band intensity were noted among different experiments but this could be due to differences in infection rates (data not shown).

Surprisingly, a protein of molecular

weight 33 KDa appeared both after the 3 days labelling period or when purified virus proteins were stained by Coomassie. This lateprotein did not appear when infectious hematopoietic necrosis virus protein synthesis was studied (Figure 1 A, B). A similar protein was found, however, when analyzing anti-VHS monoclonal antibodies by immunoblotting (Lorenzen et al, 1989) and the Kinkelin et al (1980) in purified virions. This N protein does not seem to be a contaminant since it is a protein induced by the VHS infection (Figure 1 A, B), it was immunoprecipitated by both ascites anti-VHS-144 or standard anti-VHS (F_1 + F_2 + 23.75), (Basurco and Coll, 1989), and it was detected in purified virus (Basurco and Coll, 1989 and Figure 2). Of the 2 major proteins (38 KDa and 33 KDa) seen in the N region both in the purified virions (Figure 2) and in the accumulated label (Figure 1 B), only the 38 KDa protein was labelled after a 2 h pulse. Using these short pulses a protein of intermediate molecular weight appeared stronger (see arrow Figure 1 A). A precursor-product relationship is a possible explanation for these results. A different phosphorylation pattern may also explain this heterogeneity. On the other hand, these molecules could be either viral-coded or viral-induced cellular proteins. Futher experiments are in progress tot elucidate this phenomenon. The almost complete absence of protein G in the cells after the 3 days infection (Figure 1 B) is probably due to its incorporation into the budding virus.

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Summary

We report the intracellular synthesis and the virus proteins of 5 different viral haemorrhagic septicaemia viruses isolated in Spain and compare them to the international reference strains The Spanish viruses were isolated from *Salmo gairdneri* (Richardson), *Salmo salar,* L and *Barbus graellsi* (Steindachner). The proteins detected were the L (200 KDa), G (65 KDa), N (33-38 KDa), M_1 (KDa) and M_2 (20 KDa). No differences in the molecular weight of each of these proteins was found among the Spanish isolates and the reference viruses, but in all of them the N region (33-38 KDa) was heterogeneous, containing at least 3 different proteins appearing at different times when using different pulses during infection. All these N-viral induced proteins seem to be viral since they were isolated in purified virus, and they immunoprecipitated with anti-VHS (F_1, F_2, 23.75) international antisera

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