

Identification of a new genogroup of aquareovirus by RNA–RNA hybridization

Kothandaraman Subramanian,¹ Frank M. Hetrick² and Siba K. Samal¹

¹ Virginia-Maryland Regional College of Veterinary Medicine and ² Maryland Institute for Agriculture and Natural Resources, University of Maryland, College Park, MD 20742, USA

The relative mobilities of the 11 dsRNA genomic segments of 22 aquareovirus isolates from fish and shellfish obtained from different geographical areas of the world were compared by PAGE. Using reciprocal RNA–RNA dot blot hybridization, a new sixth genetic group of aquareovirus (genogroup F) was identified. Genogroup A was represented by eight and genogroup B by 12 isolates. The remaining two isolates represented the new sixth genogroup (genogroup F). The genetic relationship of these aquareoviruses with mammalian rotavirus group A (SA11) was also examined by reciprocal RNA–RNA blot hybridization but none was found under any of the stringency conditions used.

Aquareovirus is a new genus in the family *Reoviridae* (Francki *et al.*, 1991). The virions are ether-resistant, 75 nm in diameter and consist of a double-layered capsid containing a genome composed of 11 segments of double-stranded (ds)RNA. The viruses form syncytia as a typical cytopathic effect in cell lines of fish origin (Samal *et al.*, 1990; Winton *et al.*, 1989). The absence of genetic and antigenic relatedness between aquareoviruses and the other members of the family *Reoviridae* has been reported (Samal *et al.*, 1991; Eiden & Allen, 1992). Although many isolates were obtained from normal and apparently healthy fish, some aquareoviruses have been isolated from diseased fish. Pathogenesis studies have shown that aquareoviruses can cause haemorrhagic disease and mortality in fish. The relative mobilities in polyacrylamide gels of the dsRNA segments of members of the family *Reoviridae* have been used to distinguish individual strains (Ramig *et al.*, 1977; Hrdy *et al.*, 1979; Rodger & Holmes 1979; Rodger *et al.*, 1981; Espejo *et al.*, 1980). Although the electrophoretic patterns (electropherotypes) have been used for molecular epidemiological studies (Rodger *et al.*, 1981; Estes *et al.*, 1984), they cannot be used to determine genetic relatedness among strains (Walker *et al.*, 1980; Sugiyama *et al.*, 1981). On the

other hand, reciprocal RNA–RNA hybridization has been used to study the genetic relatedness among strains of rotaviruses (Flores *et al.*, 1982; Nakagomi & Nakagomi, 1989, 1991; Nakagomi *et al.*, 1989), reoviruses (Bodkin & Knudson, 1985*a*) and orbiviruses (Bodkin & Knudson, 1986; Kowalik & Li, 1987). Initial studies using RNA–RNA hybridization showed the existence of two genetic groups among five isolates of aquareoviruses (Samal *et al.*, 1991). Using reciprocal RNA–RNA hybridization under high-, medium- and low-stringency conditions, Lupiani *et al.* (1993) identified five genogroups (designated A to E) among 19 isolates of aquareoviruses. In this study, we have analysed the electrophoretic pattern of the genomic RNA segments of an additional 22 aquareovirus isolates obtained from different geographical areas of the world and studied their genetic relatedness by reciprocal RNA–RNA hybridization. On the basis of sequence relatedness, we have identified a new sixth genetic group of aquareovirus (genogroup F). These data will be useful in studying the epidemiology, taxonomy and classification of aquareoviruses in the family *Reoviridae*.

Of the 22 aquareoviruses used in this study, 17 strains were isolated from chinook salmon (WIR, DRV, ISR, LBR, FCR, WAR, CCR, HER, SCR, IRV, KPR, MDR, SOR, HCR, ERV, LCR and NCR) and one strain each from coho salmon (SSR), chum salmon (PSR), Atlantic salmon (ASR), guppy (GRV) and smelt (SRV). A continuous cell line of chinook salmon embryo cells (CHSE) was used to propagate all the aquareovirus isolates. The simian embryonic kidney cell line MA104 was used to propagate simian rotavirus SA11. CHSE and MA104 cells were grown at 16 °C and 37 °C, respectively. Aquareoviruses, as well as SA11 virus, were purified as described elsewhere (Subramanian *et al.*, 1994). Electrophoresis of dsRNAs was performed on 7.5% polyacrylamide gels using a Tris–glycine buffer system (Laemmli, 1970). Genomic dsRNA probes were obtained from purified virus and 3'-end-labelled as described elsewhere (England & Uhlenbeck, 1978). For dot blot hybridization, denatured RNA samples were applied to a GeneScreen Plus membrane (Dupont). In order to identify sequence relatedness in the cognate genes, genomic dsRNAs extracted from different viruses were first separated on 7.5% polyacrylamide gels and then transferred to GeneScreen Plus membranes (Bodkin & Knudson, 1985*b*). Membranes were

Author for correspondence: Siba K. Samal.

Fax +1 301 935 6079. e-mail SS5@umail.umd.edu

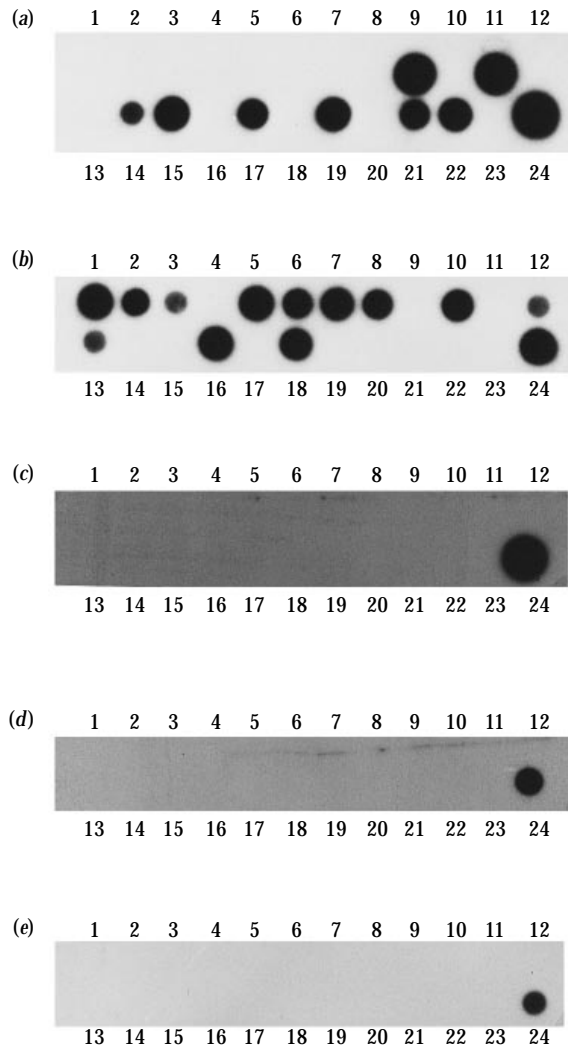


Fig. 1. Reciprocal RNA-RNA dot blot hybridization between 22 aquareoviruses and the simian rotavirus SA11 with genogroup A (SBR), genogroup B (CSR), genogroup C (GSV), genogroup D (CRV) and genogroup E (TRV). Genomic dsRNAs were extracted from purified virus, denatured by heating at 100 °C for 2 min and applied to a nylon membrane. Each probe (indicated by a to e) was made by 3'-end-labelling and hybridized to a blot containing RNAs from different viruses. (a) to (e) represent hybridizations at high-stringency conditions (hybridization at 52 °C in 50% formamide-5 × SSC, washes at 72 °C). Dots: 1, WIR; 2, DRV; 3, SSR; 4, PSR; 5, IRV; 6, KPR; 7, ISR; 8, LBR; 9, FCR; 10, MDR; 11, WAR; 12, CCR; 13, SOR; 14, HER; 15, HCR; 16, ERV; 17, GRV; 18, LCR; 19, NCR; 20, SCR; 21, ASR; 22, SRV-1.444; 23, simian rotavirus SA11. Dot 24 is represented by respective genogroups: (a) SBR, (b) CSR, (c) GSV, (d) CRV, and (e) TRV.

prehybridized for 2 h at 52 °C using hybridization buffer containing 50% formamide, 5 × SSC, 50 mM sodium phosphate pH 6.5, sonicated salmon testes DNA (100 µg/ml), 0.2% SDS and 0.1% each of BSA, Ficoll and polyvinylpyrrolidone. After overnight hybridization at 52 °C, membranes were washed at room temperature twice for 30 min in 1 × SSC-0.1% SDS and for 1 h in 0.1 × SSC-0.1% SDS at 72 °C. The membranes were then dried, sealed in plastic bags and exposed to X-ray films.

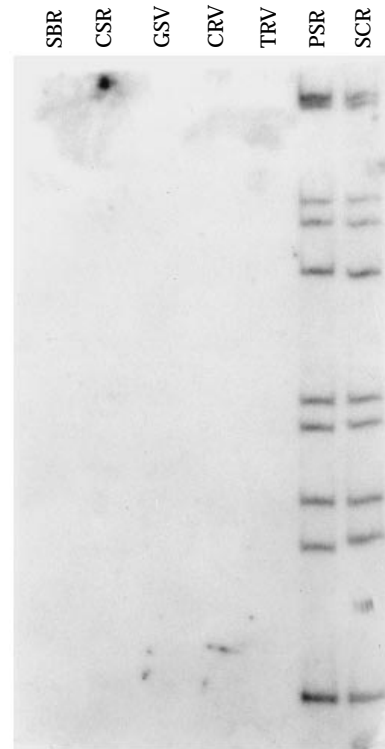


Fig. 2. Hybridization of total genomic dsRNAs of SBR (genogroup A), CSR (genogroup B), GSV (genogroup C), CRV (genogroup D), TRV (genogroup E), PSR and SCR with a 3'-end-labelled probe of SCR. Genomic dsRNAs were extracted from purified viruses, subjected to electrophoresis in 7.5% polyacrylamide gel and transferred to a GeneScreen Plus membrane. Hybridization was carried out at 52 °C, as described in the text.

The genetic relatedness of the 22 aquareovirus isolates studied by reciprocal RNA-RNA dot blot hybridization at 52 °C are shown in Fig. 1. When dsRNA from SBR isolate (genogroup A) was used as a probe, hybridization signals were observed with FCR, ERV, SOR, HER, GRV, NCR, ASR and SRV-1.444 isolates (Fig. 1a) under high-stringency conditions. When CSR (genogroup B) was used as a probe, strong hybridization signals were detected with WIR, DRV, SSR, IRV, KPR, ISR, LBR, MDR, WAR, CCR, HCR and LCR isolates (Fig. 1b) under high-stringency conditions. On the other hand, GSV (genogroup C), CRV (genogroup D) and TRV (genogroup E) isolates (Fig. 1c, d, and e) hybridized only with themselves at high-stringency conditions (Bodkin & Knudson, 1985b). Interestingly, PSR and SCR (dots 4 and 20) did not hybridize with any of the known genogroups (A to E). These results indicated that PSR and SCR could represent a new genogroup(s) in the genus *Aquareovirus*. Hybridization studies of SA11 with all the aquareovirus isolates indicated that SA11 is not genetically related to the aquareoviruses (data not shown).

Based on our dot blot hybridization studies, we found that PSR and SCR are genetically related (data not shown). To confirm our results and to further analyse the sequence

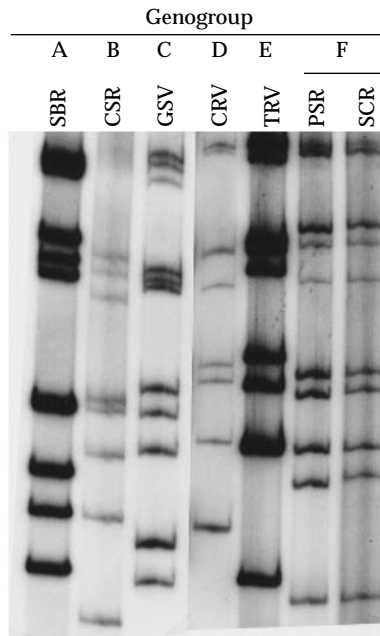


Fig. 3. Comparative genome profiles of SBR, CSR, GSV, CRV, TRV, PSR and SCR. Genomic dsRNAs were extracted from purified virus, 3'-end-labelled with [5'-³²P]pCp and subject to electrophoresis in 7.5% polyacrylamide gel. Electrophoresis was carried out for 18 h at 175 V. The genomic dsRNA segments were detected by autoradiography.

relatedness between the cognate genes of PSR and SCR viruses, genomic dsRNA segments of PSR, SCR and the known five genogroups were first separated on a polyacrylamide gel before cross hybridization at 52 °C with PSR and SCR probes. When SCR was used as a probe, hybridization was observed in all the eleven genome segments of both PSR and SCR (Fig. 2). Similar hybridization signals were also noticed when PSR was used as a probe (data not shown). These observations confirmed that PSR and SCR are genetically related and represent a new sixth genogroup, designated F. The electrophoretic patterns of the five established genogroups A to E (SBR, CSR, GSV, CRV and TRV) and the two isolates in the new genogroup F (PSR and SCR) are shown in Fig. 3.

In this study, 22 aquareovirus isolates were used to study their genetic relatedness by reciprocal RNA-RNA hybridization. Based on these hybridization studies, a sixth genetic group (genogroup F) has been established. Cross hybridization of electrophoretically separated genome segments showed that the members of genogroup F had sequence relatedness in all cognate genes. Within rotaviruses, viruses belonging to one genogroup are usually derived from the same animal species (Nakagomi & Nakagomi, 1991). This is not true for aquareoviruses; viruses belonging to genogroups A, B or F were recovered from different species of fish. Even though there are two viruses in genogroup F, it was interesting to find that they were isolated from two different hosts (chum and chinook salmon). Lupiani *et al.* (1993) found a correlation between genogroups and the location of isolation, suggesting the

possibility of a common origin such as the source of food for these species. In this study, it was found that all viruses belonging to genogroup B were isolated from Washington, USA. Viruses belonging to genogroup A have been isolated from a wide range of geographical areas. GRV, an isolate from Singapore, hybridized strongly with genogroup A. The two isolates in genogroup F were isolated from Washington and Alaska. Further studies involving the isolation of aquareoviruses will be useful to better understand the origin and epidemiology of the different aquareovirus genogroups.

The data obtained in this study confirm and extend our previous results (Lupiani *et al.*, 1993), which concluded that there is wide diversity in the genus *Aquareovirus*, and provide an experimental criterion for dividing aquareoviruses in to genogroups. Reassortment of aquareovirus among genogroups does not seem to occur (unpublished data). However, the possibility of reassortment among strains of a genogroup in nature cannot be ruled out at present. The genogroups determined in this study could be used for comparison with new aquareoviruses as they are isolated. Further biological and serological studies on the isolates in each genogroup are required before the divisions made here can be fully substantiated.

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