Pestiviruses—taxonomic perspectives

M. C. Horzinek

Dept. Infect. Dis. & Immunol., Veterinary Faculty, Institute of Virology, Utrecht,
The Netherlands

Accepted March 14, 1991

Summary. The history of pestivirus taxonomy is surprisingly consistent: almost 30 years ago it was recognized that pestiviruses are structurally akin to flaviviruses, and recent nucleotide sequence data have confirmed this resemblance at the level of genome organization. For other enveloped positive stranded RNA viruses with icosahedral nucleocapsids e.g. equine arteritis and lactate dehydrogenase virus of mice a taxonomic dilemma is encountered; while virions resemble (“non-arthropod-borne”) togaviruses, the replication via a nested set of subgenomic RNAs is corona and torovirus-like. Pestiviruses, flaviviruses and the hepatitis C virus group have been assigned generic status in the Flaviviridae family.

Key words: Pestivirus, taxonomy.

For a long time, viral taxonomy was based exclusively on structural features of the infectious particle. Three alternative criteria were used: the type of nucleic acid (DNA or RNA), the lipoprotein envelope (present or absent) and the nucleocapsid symmetry type (icosahedral or helical). While the first two features of a virus are easily established—using nucleic acid inhibitors and organic solvents or detergents, respectively—identification of the symmetry type in enveloped virions is less straightforward. It involves electron microscopic analysis after selective removal of the virion membrane, an approach that has led to the identification of icosahedral capsids in the arthropod-borne alphaviruses [10] and in other, then unclassified small enveloped viruses without notorious arthropod transmission [11]. These included rubella virus (which has remained in the Togaviridae family), the pestiviruses discussed in this volume, and the equine arteritis/lactic dehydrogenase viruses.

In recent years, there is a tendency to abandon strictly structural criteria of classification and to make use of other properties, e.g. the replication strategy of viruses. This is a rational approach if taxonomy is to reflect evolutionary relationships. After all, more constraints, more selective pressure may be expected to operate at the level of the extracellular virus particle, its proteins and antigenic determinants than e.g. at the level of mRNA transcription; the mode of replication is probably more conserved during evolution.

In 1973, I have coined the term “pestiviruses” to congregate two antigenically related enveloped RNA viruses: hog cholera virus (HCV) and BVDV [8]. A third animal pathogen, the border disease virus of sheep, was found later to be a close relative of BVDV. In its Fourth Report published in 1982 for the Virology Division of the International Union of Microbiological Societies (IUMS), the International Committee on Taxonomy of Viruses (ICTV) has adopted this nomenclature and has assigned generic status to the pestiviruses, with BVDV as the prototype [12]. Pestiviruses (from lat. *pestis suum* = hog cholera or ‘classical’ swine fever, an economically important disease) are amongst the smallest enveloped animal RNA viruses (measuring about 40 nm in diameter) and possess a nucleocapsid of non-helical, probably icosahedral symmetry [9]. They share these traits with the numerous flaviviruses, of which the mosquito-transmitted yellow fever virus is the prototype (the pestiviruses are non-arthropod-borne). Previously, also the flaviviruses held generic status in this family, but when details of their molecular structure, replication strategy and gene sequence became known in the early 1980s, the Togavirus Study Group acknowledged the differences as fundamental and established the new family Flaviviridae—with flavivirus then its only genus [20].

In view of the recent progress in the description of the molecular features of pestiviruses, the discussion of virus classification has been re-opened. The first molecular data which suggested that pestiviruses are distinct from members of the Togaviridae family relate to characteristics of the virus-specific RNA. In infected cells, only a single high molecular weight species was found which lacked a 3′ poly(A) tract [14, 15; R. Moormann, personal communication]. No subgenomic RNA was detected at any time after infection. These properties distinguish pestiviruses from togaviruses—of which both the alpha- and rubiviruses possess one subgenomic RNA—and suggest a similarity to flaviviruses. Much of the nucleotide sequence and genetic organization of BVDV is known, and further comparisons with flaviviruses have been made [2]. With the exception of several short but significant stretches of identical amino acids within two of the putative nonstructural proteins, no extended regions of homology exist between BVDV and representatives of the three antigenic subgroups of mosquito-borne flaviviruses. Nevertheless, the molecular layout of the BVDV and flavivirus genomes is strikingly similar. Comparison of the arrangement of

the protein coding domains along both genomes and the hydropathic features of their amino acid sequences revealed pronounced similarities. Based on these comparisons, it was suggested that the Pestiviruses no longer be grouped in the Togaviridae family, but rather be considered a genus within the Flaviviridae [2]. A joint proposal by the Togavirus and Flavivirus Study Groups read: "To remove the genus Pestivirus from the Family TOGAVIRIDAE and place it in the family FLAVIVIRIDAE." This proposal has been accepted at the last assembly of the ICTV during the VIIIth International Congress of Virology in Berlin. In the forthcoming Report of the ICTV (Francki et al., 1991, in press) a third virus will be found allotted to the FLAVIVIRIDAE: the recently cloned and sequenced hepatitis C virus. Although neither visualized by electron microscopy nor analyzed by conventional means, the 7310 base genome shows an organization akin to flaviviruses [1]. It can be expected that additional members of animal 'hepatitis C virus group' members will be discovered.

The implications of this proposition are immediately provocative. Considering the parallel organization of their genomes, analogous polypeptides may be predicted to possess similar biologic functions. Examples corroborating this hypothesis have been given in our recent review on molecular advances in pestivirus research [3]. Drawing analogies to the flaviviruses may help in experimental designs to resolve the issue of structural vs. nonstructural proteins for pestiviruses. Certainly insights beyond those in molecular biology may be gained from further comparisons between the three groups of viruses (flavi-, pesti- and hepatitis C-viruses). Thus the aspect of human infections with pestiviruses has recently obtained special attention. In 41% to 87% of human sera from Europe, Africa, Central and South America antibodies against BVDV have been found in persons with frequent contacts to bovines [19]; the evidence has been confirmed by antigen detection and by polymerase chain reaction amplification of pestiviral sequences in faeces from children with gastrointestinal disease [21].

Non-arthropod-borne togaviruses were last reviewed in 1981 (with flaviviruses at that time still belonging to the Togaviridae family), when virtually no molecular data were available [9]. Meanwhile, the expanding knowledge has made yet another morphological classification untenable: equine arteritis virus (EAV) which has been listed as a possible member of the Togaviridae family [12] replicates via multiple subgenomic mRNAs [18]; we have recently found that they form a 3'-coterminal nested set [4], like in coronaviruses and toroviruses [16]. However, by having the ≤ 70 nm size and possessing an enveloped icosahedral nucleocapsid, EAV does fulfill conventional structural criteria of a togavirus. Equine arteritis virus is morphologically and structurally similar to lactic dehydrogenase virus (LDV [9]) and also its genome organization was recently found to be quite similar [6]. In all three groups, the coronaviruses, toroviruses and arteriviruses the polymerase gene contains two overlapping open reading frames

which are expressed as a fusion protein by a process of ribosomal frame-shifting; the predicted tertiary RNA structure (a pseudoknot) in the frameshift-directing region is similar. The data obtained in Utrecht on the organization and translation of the polymerase gene of Berne virus (the torovirus prototype) provide additional evidence that toro-, corona- and arteriviruses are evolutionarily more related to each other than to any other family of positive-stranded RNA viruses. They can be considered to form a third 'superfamily' [7, 17], perhaps an order, as has recently been decided by the ICTV for three families of negative-stranded RNA viruses: the paramyxo-, rhabdo- and filoviridae (Order Mononegavirales [13]).

The presence of "incompatible" taxonomic elements in one virus (e.g. EAV showing the togavirus characteristics of an icosahedral capsid together with the nested set of subgenomic RNAs of the third 'superfamily') indicates that our traditional concept of a structural classification of viruses is certainly too narrow. In retrospect, it must be considered fortuitous that modern analytic methods have confirmed what morphology and scarce physico-chemical data have suggested already in the 1960s [5]: that pestiviruses are flavivirus-like.

Acknowledgements

The author should like to thank Marc Collett and Volker Moennig for many discussions and suggestions, most of which have been included in a recent review article on pestiviruses [3].

References

1. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M (1989) Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 244: 359–362
2. Collett MS, Anderson DK, Retzel E (1988) Comparisons of the pestivirus bovine viral diarrhoea virus with members of Flaviviridae. *J Gen Virol* 69: 2637–2643
3. Collett MS, Moennig V, Horzinek MC (1989) Recent advances in pestivirus research. *J Gen Virol* 70: 253–266
4. De Vries AAF, Chirnside ED, Bredenbeek PJ, Gravestien LA, Horzinek MC, Spaan WJM (1990) All subgenomic mRNAs of equine arteritis virus contain a common leader sequence. *Nucl Acids Res* 18: 3241–3247
5. Dinter Z (1963) Relationship between bovine virus diarrhoea virus and hog cholera virus. *Zentralbl Bakteriol Parasitenk Abt 1 Orig* 188: 475–486
6. Godeny EK, Brinton MA (1990) Sequence analysis and genome organization of lactate dehydrogenase-elevating virus (LDV) Abstracts VIII Intern Cong Virol Berlin P52-016: 376
7. Goldbach R, Wellink J (1988) Evolution of plus-stranded RNA viruses. *Intervirology* 29: 260–267
8. Horzinek MC (1973) The structure of togaviruses. *Prog Med Virol* 16: 109–156
9. Horzinek MC (1981) Non-arthropod-borne togaviruses. Academic Press, London
10. Horzinek MC, Mussgay M (1969) Studies on the nucleocapsid structure of a group A arbovirus. *J Virol* 4: 514–520

11. Horzinek MC, Maess J, Laufs R (1967) Studies on the substructure of togaviruses. II. Analysis of equine arteritis, rubella, bovine viral diarrhoea and hog cholera viruses. *Arch Ges Virusforsch* 33: 306–318
12. Matthews REF (1982) Classification and nomenclature of viruses. 4th Report of the International Committee on Taxonomy of Viruses. Karger, Basel
13. Pringle CR (1991) The order Mononegavirales. *Arch Virol* 117: 137–140
14. Purchio AF, Larson R, Collett MS (1983) Characterization of virus-specific RNA synthesized in bovine cells infected with bovine viral diarrhoea virus. *J Virol* 48: 320–324
15. Renard A, Guiot C, Schmetz D, Dagenais L, Pastoret P, Dina D, Martial JA (1985) Molecular cloning of bovine diarrhoea viral diarrhoea virus sequences. *DNA* 4: 429–438
16. Snijder EJ, Ederveen J, Spaan WJM, Weiss M, Horzinek MC (1988) Characterization of Berne virus genomic and messenger RNAs. *J Gen Virol* 69: 2135–2144
17. Strauss JH, Strauss EG (1988) Evolution of RNA viruses. *Ann Rev Microbiol* 42: 657–683
18. Van Berlo MF, Spaan WJM, Horzinek MC (1987) Replication of equine arteritis virus (EAV): a comparative review. In: *The molecular biology of the positive strand RNA Viruses*. Academic Press, London, p 105–115
19. Verhulst A, Van Opdenbosch E, Wellemans G, Giangaspero M (1988) Susceptibility of man to bovine viral diarrhoea virus. *Lancet* ii: 110
20. Westaway EG, Brinton MA, Gaidamovich SYA, Horzinek MC, Igarashi A, Kääriäinen L, Lvov DK, Porterfield JS, Russell PK, Trent DW (1985) Flaviviridae. *Intervirology* 24: 183–192
21. Yolken R, Leister F, Eiden J (1990) RNA analyses of pestivirus isolated from humans. *Abstracts VIII Intern Cong Virol Berlin*, W21-002: 50

Author's address: Prof. Dr. M. C. Horzinek, Institute of Virology, Dept. Infect. Dis. & Immunol., Veterinary Faculty, Yalelaan 1 (de Uithof), 3508 TD Utrecht, The Netherlands.