

# Picornavirus Classification Below the Level of Species: New Serotypes or Types

Nick J. Knowles<sup>1</sup>, M. Steven Oberste<sup>2</sup>, Mark A. Pallansch<sup>2</sup>, Glyn Stanway<sup>3</sup> and Tapani Hovi<sup>4</sup>

1) Institute for Animal Health, Pirbright Laboratory, Pirbright, Surrey, UK; 2) Enterovirus Team, CDC, Atlanta, USA; 3) Dept. of Biological Sciences, University of Essex, UK; 4) Enterovirus Laboratory, KTL, Finland.

## ABSTRACT

At EUROPIC 2000 we posed a number of questions with regard to new picornavirus serotypes, such as which criteria should be used for serotype definition and who should make decisions concerning future serotype nomenclature and designation. In the intervening years a system has been put in place whereby researchers submit data on candidate serotypes (or types) to the chairman of the Picornaviridae Study Group for approval. It has been decided that new types can be designated based on sequence data alone, however, it may be preferable to have some supporting serological data. The majority of new types that have been approved are within the genus *Enterovirus* and currently consist of enterovirus (EV) 73 to EV-102. However, viruses in two other genera pose some interesting problems. In the genus *Parechovirus*, three serologically distinct human parechovirus (HPeV) serotypes are recognised three more have recently been proposed; however, two isolates of HPeV-2 are genetically very diverse and should represent distinct types. In the genus *Erbovirus*, some strains of a newly recognised equine rhinitis B virus (ERBV) serotype (ERBV-3) appear to cross-react antigenically with ERBV-1 and, in one isolate, intertypic recombination between the two serotypes has been reported within capsid region. Genetic criteria for the classification of types within each species still need to be clarified (in the case of enteroviruses) or established (for most other picornaviruses). The current situation will be presented. Pages within the new Picornaviridae Study Group website have been constructed which present the new picornavirus type designations: [www.picornaviridestudygroup.com/typelist/](http://www.picornaviridestudygroup.com/typelist/).

## METHODS

Phylogenetic (Neighbor-joining) trees were constructed using MEGA 3.1 (Kimura 2-parameter model) (Kumar et al., 2004). Confidence limits on branching were assessed using bootstrap re-sampling (1000 pseudo-replicates) and are shown as percentages.

## PARACHOVIRUSES

The complete genome sequences of four new human parechovirus serotypes have recently been described (Table 2). The isolate 86-6760, previously classified as HPeV-2 due to a serological cross-reaction with HPeV-2 Williamson (the prototype strain), has now been designated as HPeV-5 because of its genetic distinctiveness. A new genome sequence has recently been deposited in the GenBank/EMBL/DBJ databases as HPeV-6 (isolate BNI-7885; accession EF051629); however, the P1 region of this sequence is closely related to the newer HPeV-1 viruses and not the newly designated HPeV-6 (Table 2; Fig. 2). Therefore its classification deserves further scrutiny. The shaded bar indicating a range of amino acid differences that divide the human parechovirus types is shown in Fig. 2. If this level of difference was applied to the Ljungman viruses then two or three types could be defined. Further studies are necessary.

Table 2. Details of the new human parechovirus types.

New type	Strain ID	Species	Proposer	Supporting data	Accession	Reference(s)
HPeV-3	A308/99	HPeV	M. Ito	CG	AB04913	Ito et al., 2004
	Can82853-01	HPeV	AJ889918	CG	AJ889918	Abed and Bovin, 2005
HPeV-4	K251176-02	HPeV	Kimberley Benschop	CG	DQ315670	Benschop et al., 2006
	T75-4077	HPeV	Al-Sunaidi et al., 2006	CG	AM235750	Al-Sunaidi et al., 2006
HPeV-5	86-6760*	HPeV	Glyn Stanway	CG	AF055846	Oberste et al., 1998
	192-15	HPeV	Glyn Stanway	CG	AM235749	Al-Sunaidi et al., 2006
HPeV-6	NI0561-2000	HPeV	Masahiro Fujii	CG	AB252582	Watanabe et al., unpublished

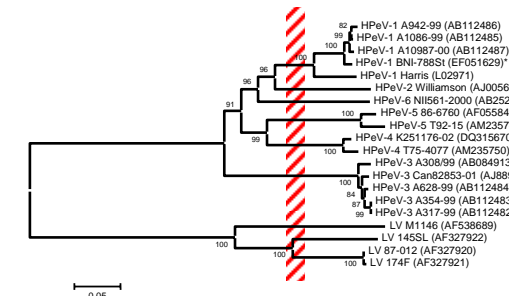


Fig. 2. Mid-point rooted Neighbor-joining tree of the P1 capsid (aa) of parechoviruses. The shaded bar indicates a range of amino acid differences that divide the human parechovirus types. LV, Ljungman virus. \*, labelled at HPeV-6 on GenBank/EMBL/DBJ.

## ANTIGENIC MIMICRY – antigenic cross-reactions are not always reliable indicators of genetic relationships

An antigenic cross-reaction between encephalomyocarditis virus (Picornaviridae) and cricket paralysis virus (*Dictyostelidae*) has been reported and it was even proposed that these were the same virus (Tinsley et al., 1984). Cooney et al. (1973, 1982) demonstrated low-level cross-neutralization between various human rhinovirus serotypes; however, now that molecular sequence data is available, it is evident that some of these cross-reactions were not phylogenetically based, e.g. HRV-48 and HRV-55, which belong to different HRV species. It is also predicted that viruses containing RGD-integrin-binding loops may cross-react and possibly even cross-neutralize since it is known that neutralizing FMDV monoclonal antibodies recognize this region (Fig. 4; N.J. Knowles, unpublished observations). Also, it has long been recognised that certain isolates of FMDV serotype SAT3 may be neutralized, *in vitro*, by antisera to FMDV serotype SAT1 (unpublished observations). The basis for this cross-reaction is not understood, but similarities between the sequences of SAT1 and SAT3 (but not SAT2) at the carboxy-terminal end of VP1 could account for this observation (N.J. Knowles, unpublished observations). It would therefore appear that antigenic cross-reactions are not always reliable indicators of phylogenetic relationships.

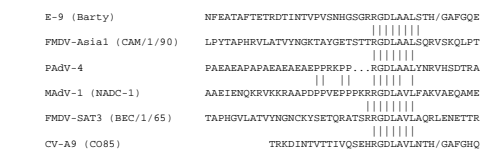


Fig. 4. Alignment of the RGD-motif regions of diverse viruses. E-9, echovirus 9 VP1/2A; FMDV, foot-and-mouth disease virus VP1 G-H loop; PAdV-4, porcine adenovirus 4 fibre knob; MdV-1, mouse adenovirus 1 fibre knob; CV-A9, coxsackievirus A9 VP1/2A.

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## ENTEROVIRUSES

Table 1 lists the new human enterovirus (EV) serotypes designated EV-73 to EV-102. Most (=20) new serotypes fall within the species *Human enterovirus B* (yellow) and *Human enterovirus A* (blue), *Human enterovirus C* (purple) and *Human enterovirus D* (green) are represented by 5, 4 and 1 serotypes, respectively. The relationships between their VP1 sequences are depicted in Fig. 1.

Table 1. Proposed new enterovirus types.

New type	Strain ID	Species	Proposer	Supporting data	Accession	Reference(s)
EV-73	CA56-1988	HEV-B	Mark Pallansch	complete genome	AF241359	Oberste et al., 2001
EV-74	USA/CA75-10213	HEV-B	Mark Pallansch	complete genome	AY556657	Oberste et al., 2004
EV-75	USA/OK85-10362	HEV-B	Mark Pallansch	complete genome	AY556670	Oberste et al., 2004
EV-76	FRA91-10369	HEV-A	Steve Oberste	complete genome	AY697458	Oberste et al., 2005
EV-77	CF496-99	HEV-B	Helene Norder	complete genome	AJ493062	Bailey et al., unpublished
	USA/TX97-10394	HEV-B	Helene Norder	complete genome	AY843302	Oberste et al., unpublished
EV-78	Agilus_W137-12699	HEV-B	Helene Norder	P1	AY208120	Norder et al., 2003
EV-79	USA/CA79-10384	HEV-B	Steve Oberste	complete genome	AY945297	Oberste et al., unpublished
EV-80	USA/CA67-10387	HEV-B	Steve Oberste	complete genome	AY945298	Oberste et al., unpublished
EV-81	USA/CA68-10389	HEV-B	Steve Oberste	complete genome	AY945299	Oberste et al., unpublished
EV-82	USA/CA64-10390	HEV-B	Steve Oberste	complete genome	AY943300	Oberste et al., unpublished
EV-83	USA/CA76-10392	HEV-B	Steve Oberste	complete genome	AY943301	Oberste et al., unpublished
EV-84	10603	HEV-B	Steve Oberste	VP1	n/a	Oberste et al., unpublished
EV-85	BAN00-10353	HEV-B	Steve Oberste	complete genome	AY843303	Oberste et al., unpublished
EV-86	BAN00-10354	HEV-B	Steve Oberste	complete genome	AY843304	Oberste et al., unpublished
EV-87	BAN01-10396	HEV-B	Steve Oberste	complete genome	AY843305	Oberste et al., unpublished
EV-88	BAN01-10398	HEV-B	Steve Oberste	complete genome	AY843306	Oberste et al., unpublished
EV-89	BAN00-10359	HEV-A	Steve Oberste	complete genome	AY697459	Oberste et al., 2005
	BAN00-10399	HEV-A	Steve Oberste	complete genome	AY697460	Oberste et al., 2005
EV-90	F950027 (Netherlands)	HEV-A*	Steve Oberste	complete genome	AY773285	van den Broek et al., unpublished
	CAM1956 (Cambodia)	HEV-A*	Steve Oberste	complete genome	AB192877	Shimizu et al., unpublished
EV-91	BAN00-10406	HEV-A*	Steve Oberste	complete genome	AY697461	Oberste et al., 2005
EV-92	GA01-10263	HEV-A	Steve Oberste	VP1	n/a	Oberste et al., unpublished
EV-93	38_03	HEV-B	Helene Norder	VP1	n/a	Norder et al., unpublished
EV-94	EGY2003	HEV-D	Merja Rovainen	VP1	n/a	Smura et al., submitted for publication
EV-95	E-06	HEV-C	Helene Norder	VP1	n/a	Norder et al., unpublished
	SV003-24	HEV-C	Merja Rovainen	VP1	n/a	Smura et al., unpublished
EV-96	10488	HEV-C	Merja Rovainen	VP1	AY191872	Oberste et al., 2006
	10548c	HEV-C	Merja Rovainen	partial VP1	AY191838	Oberste et al., 2006
EV-97	BAN09-10355	HEV-B	Merja Rovainen	complete genome	AY843307	Oberste et al., unpublished
EV-98	92-1499	HEV-B	Teruo Yamashita	VP1	n/a	Yamashita et al., unpublished
EV-99	10461-ban00	HEV-C	Mark Pallansch	VP1	n/a	Brown et al., unpublished
EV-100	BAN00-10410	HEV-B	Steve Oberste	VP1	n/a	Oberste et al., unpublished
	10500	HEV-B	Steve Oberste	VP1	AY191848	Oberste et al., 2006
EV-101	CI003-10361	HEV-B	Steve Oberste	complete genome	AY843308	Oberste et al., 2006
EV-102	10424-BAN99	HEV-C	Mark Pallansch	VP1	n/a	Brown et al., unpublished

## ERBOVIRUSES

Two new equine rhinitis B virus (ERBV) serotypes have recently been identified. The complete genome sequence of ERBV-2 (formerly known as equine rhinovirus 3) was determined (see Table 3). This virus was already recognised a being serologically distinct from ERBV-1 (formerly known as equine rhinovirus 2) (Steck et al., 1978). Recently, part of the 3' UTR and P1 sequences of an acid-stable equine picornavirus have been determined and proposed as ERBV-3 (see Table 3). However, a number of ERBV-3 isolates cross-react with ERBV-1 antisera. The basis for this is unknown, however, one virus has been isolated which has an ERBV-1/ERBV-3 recombinant capsid (Black et al., 2005).

Table 3. Proposed new equine rhinitis B virus types.

New type	Strain ID	Species	Proposer	Supporting data	Reference(s)
ERBV-2	P313/75	ERBV	Michael Studt	CG	AF361253 Huang et al., 2001
ERBV-3	4442/75	ERBV	Nick Knowles	partial 3D & 3' UTR	n/a Knowles, 2005
	R4/75	ERBV	Nick Knowles	P1	DQ108383 Black and Studt, 2006
	2484c/75	ERBV	Nick Knowles	P1	DQ108384 Black and Studt, 2006
	379/75	ERBV	Nick Knowles	P1	AY060992 Black et al., 2005
	831/1189	ERBV	Nick Knowles	P1	AY060996 Black et al., 2005
	9051-789	ERBV	Nick Knowles	P1	AY060997 Black et al., 2005

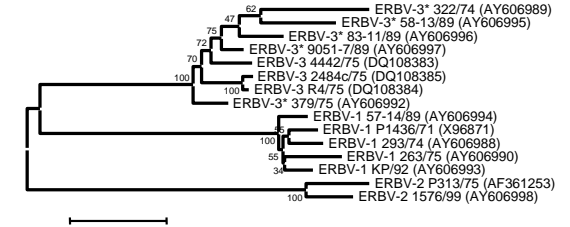


Fig. 3. Mid-point rooted Neighbor-joining tree of the P1 capsid (aa) of erboviruses. \* these ERBV-3 viruses appear to have an antigenic cross-reaction with ERBV-1.

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## CONCLUSIONS

Due to the logistical difficulties of raising antisera against large numbers of viruses it is not always possible to conduct cross-neutralization tests on potential new serotypes (particularly with the entero- and rhinoviruses). Since it is now relatively simple to obtain sequence data on new viruses, we propose that picornavirus groupings below the species level are based on genetic groupings and are called **types** rather than **serotypes**. However, the criteria used to designate new types needs to be reviewed. Studies on the molecular diversity of VP1 sequences with the established serotypes may help to clarify the situation.

Proposals for new serotypes should be sent, with supporting data, to the Chair of the Picornaviridae Study Group who will designate the new type numbers.