# Molecular and Biochemical Analysis of Bovine Rhinovirus Type 2 Show a Close Relationship to the Aphthoviruses


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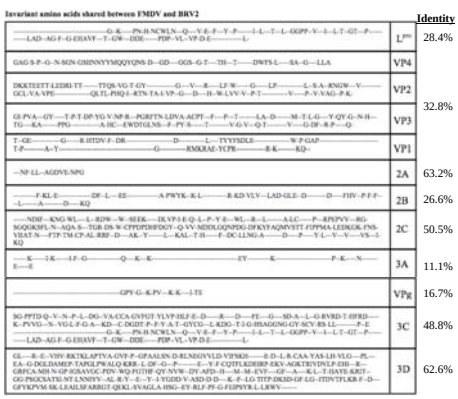
## Introduction

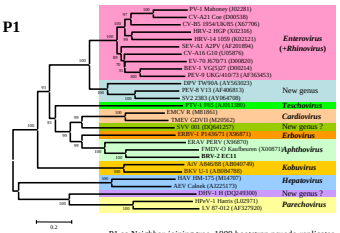
- Bovine rhinovirus 2 (BRV-2) was first isolated circa 1964 by Reed et al. and is currently an unclassified species tentatively assigned to the genus *Aphthovirus* in the family *Picornaviridae*.
- Bovine rhinoviruses have been found in the upper respiratory tract of animals with acute respiratory disease and from nasal swabs of apparent healthy animals.
- Previous analysis of small regions encoded by BRV2 showed a high similarity with foot-and-mouth disease viruses (Knowles; EUROPIC 2005).
- In the current study we have extended previous observations by isolating an almost full-length cDNA encoding the genome of BRV-2 and performing bioinformatics analysis on the sequence and *in vitro* characterization of the viral proteins.

**Alignment of BRV-2 and FMDV amino acid sequences reveals a high level of conservation**

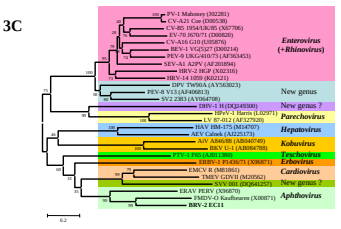



The invariant amino acids table was derived by aligning the BRV-2 amino acid consensus with the FMDV amino acid consensus that was generated for each serotype from 133 FMDV. Invariant amino acids are given in one letter code.

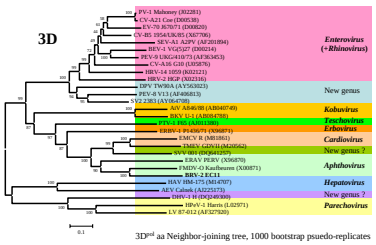
**Neighbor-joining tree showing the relationship of the BRV-2 P1, 3C and 3D amino acid sequence to other members of the family *Picornaviridae***




P1 aa Neighbor-joining tree, 1000 bootstrap psuedo-replicates

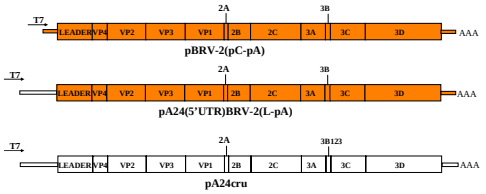



3C$^{pro}$ aa Neighbor-joining tree, 1000 bootstrap psuedo-replicates

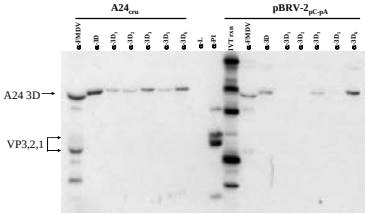



3D$^{pol}$ aa Neighbor-joining tree, 1000 bootstrap psuedo-replicates

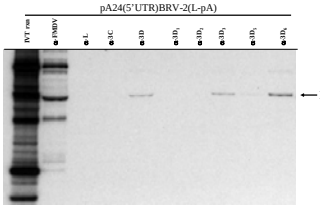
**Cloning and characterization of expression constructs containing just the BRV-2 genome (polyC to the polyA) or BRV-2 (L-pA) fused to FMDV (5'-UTR)**

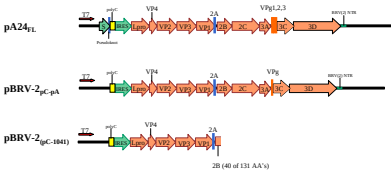



**Radioimmunoprecipitation of BRV-2 proteins with FMDV antibodies**

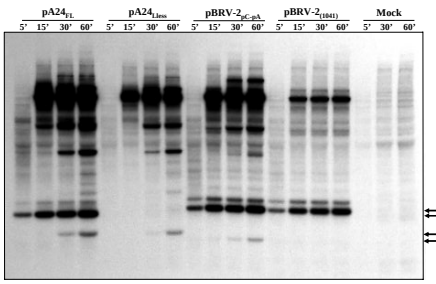



BRV-2 proteins expressed and processed in BHK-S10 cell extracts are recognized by anti-sera against FMDV.



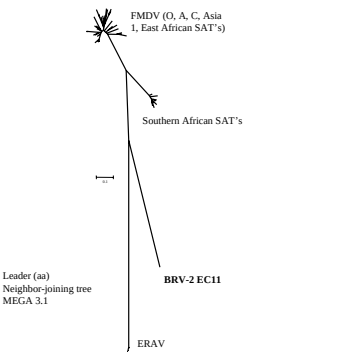

**Analysis of polyprotein processing in BHK-S10 cell extracts at early time points**



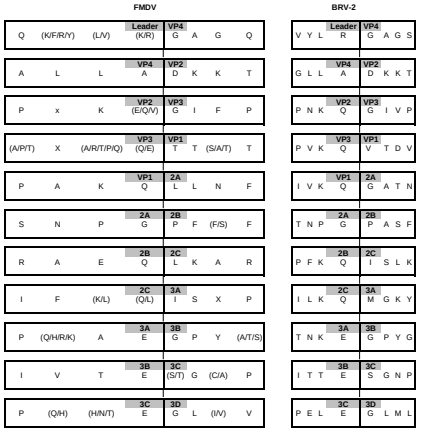

A protein migrating at the expected size for BRV-2 leader protein is released at very early times of protein synthesis.

**Comparison of Picornavirus VPgs**

| Genotype | VPg amino acid sequence |
|---|---|
| PV | G A Y T G L . P N K K P N V P T I R T A K V Q |
| HRV14 | G A Y S G N P P H N K L K A P T L R P V V V Q |
| HRV2 | G A Y S G E . P K P K T K I P E . R P V V T Q |
| HRV89 | G A Y S G E . P K P K S R A P E . R V V T Q |
| BRV-2 | G P Y G G Q A K P V K V D K L K V N P L I T T E |
| FMDV VPg1 | G P Y A G P L E R Q K P L K V R A K L P Q Q E |
| FMDV VPg2 | G P Y A G P M E R Q K P L K V K A K A P V V K E |
| FMDV Vpg3 | G P Y E G P V K K P V A L K V K A K N L I V T E |
| ERV-2 | S A Y D P S T K P K P K T Q E V K T L K I R T E |

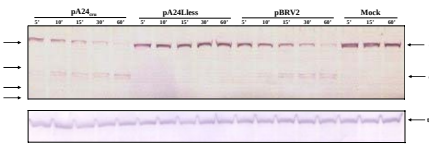



**Predicted proteolytic cleavage sites based on polyprotein junctions**




| Genus | Species | Species | Serotype | Serotype |
|---|---|---|---|---|
| *Enterovirus* | PV | Poliovirus | PV-1 | Poliovirus 1 |
| | HEV-A | *Human enterovirus A* | CV-A16 | Coxsackievirus A16 |
| | HEV-B | *Human enterovirus B* | CV-B5 | Coxsackievirus B5 |
| | HEV-C | *Human enterovirus C* | CV-A21 | Coxsackievirus A21 |
| | HEV-D | *Human enterovirus D* | EV-70 | Enterovirus 70 |
| | SEV-A | *Simian enterovirus A* | SEV-A1 | Simian enterovirus A1 |
| | BEV | *Bovine enterovirus* | BEV-1 | Bovine enterovirus 1 |
| | PEV-B | *Porcine enterovirus B* | PEV-9 | Porcine enterovirus 9 |
| *Rhinovirus* | HRV-A | *Human rhinovirus A* | HRV-2 | Human rhinovirus 2 |
| | HRV-B | *Human rhinovirus B* | HRV-14 | Human rhinovirus 14 |
| *Cardiovirus* | EMCV | *Encephalomyocarditis virus* | EMCV | Encephalomyocarditis virus |
| | TMV | *Theilovirus* | TMEV | Theiler's murine encephalomyelitis virus |
| *Aphthovirus* | FMDV | *Foot-and-mouth disease virus* | FMDV-O | Foot-and-mouth disease virus O |
| | ERAV | *Equine rhinitis A virus* | ERAV | Equine rhinitis A virus |
| | | *unnamed* | BRV-2 | *Bovine rhinovirus* |
| *Hepatovirus* | HAV | *Hepatitis A virus* | HAV | Hepatitis A virus |
| | AEV | *Avian encephalomyelitis virus* | AEV | Avian encephalomyelitis virus |
| *Parechovirus* | HPeV | *Human parechovirus* | HPeV-1 | Human parechovirus 1 |
| | LV | *Ljungan virus* | LV | Ljungan virus |
| *Erbovirus* | ERBV | *Equine rhinitis B virus* | ERBV-1 | Equine rhinitis B virus 1 |
| *Kobuvirus* | AiV | *Aichi virus* | AiV | Aichi virus |
| | BKV | *Bovine kobuvirus* | BKV | Bovine kobuvirus |
| *Teschovirus* | PTV | *Porcine teschovirus* | PTV-1 | Porcine teschovirus 1 |
| *"Sapelovirus"* | PEV-A | *Porcine enterovirus A* | PEV-8 | Porcine enterovirus 8 |
| | | *unnamed* | SV2 | Simian virus 2 |
| | | *unnamed* | DPV | Duck picornavirus |
| New genus? | | *unnamed* | SVV | Seneca Valley virus |
| New genus? | | *unnamed* | DAV-1 | Duck hepatitis virus 1 |

**Analysis of eIF4G cleavage in LFBK cell extracts programed with BRV-2 RNA**




eIF4G is processed to a lower molecular weight form in cell extracts programmed with BRV-2 RNA, similar or identical to what occurs with FMDV.

## Summary:

- A more extensive bioinformatics analysis of BRV-2 confirms previous observations that BRV-2 is closely related to foot-and-mouth disease viruses.
- BRV-2 encodes a leader protein that in these preliminary experiments appears to have a protease activity capable of autocatalyzing its release from P1 and functions in the cleavage of eIF4G.
- Strong similarities in the non-structural proteins revealed by sequence analysis were partially confirmed by cross-reactivity of BRV-2 3D with FMDV anti-3D.
- Further characterization of BRV-2 viral proteins as well as isolation of the entire 5'-UTR is currently underway.