

# Discovery of a Novel Porcine Bastrovirus Associated with Non-Suppurative Meningoencephalomyelitis in Pigs

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## Background

Three growing-finisher pigs from two farms showing neurological signs were submitted for post-mortem examination to the Institute of Animal Pathology Bern. Histopathological examination revealed a severe lympho-histiocytic meningoencephalomyelitis. Further (RT-) PCR testing failed to identify any routinely tested virus as the cause of disease. In order to identify the associated agent, high throughput sequencing (HTS) was performed from RNA extracted from the spinal cord. This step was followed by bioinformatics-based virus discovery. We obtained an almost full genome of a new porcine bastrovirus strain (PoBastV-CH22) and through rapid amplification of cDNA ends (RACE) we could complete it. Bastroviruses are found in human and animal fecal samples<sup>1,2,3</sup>. To date, no association of bastrovirus infection and neurological disease has been described. However, the related hepeviruses and astroviruses are known to induce severe disease in humans and animals. Especially astroviruses are a known cause of non-suppurative encephalitis in immunocompromised human patients and in animal species<sup>4</sup>.

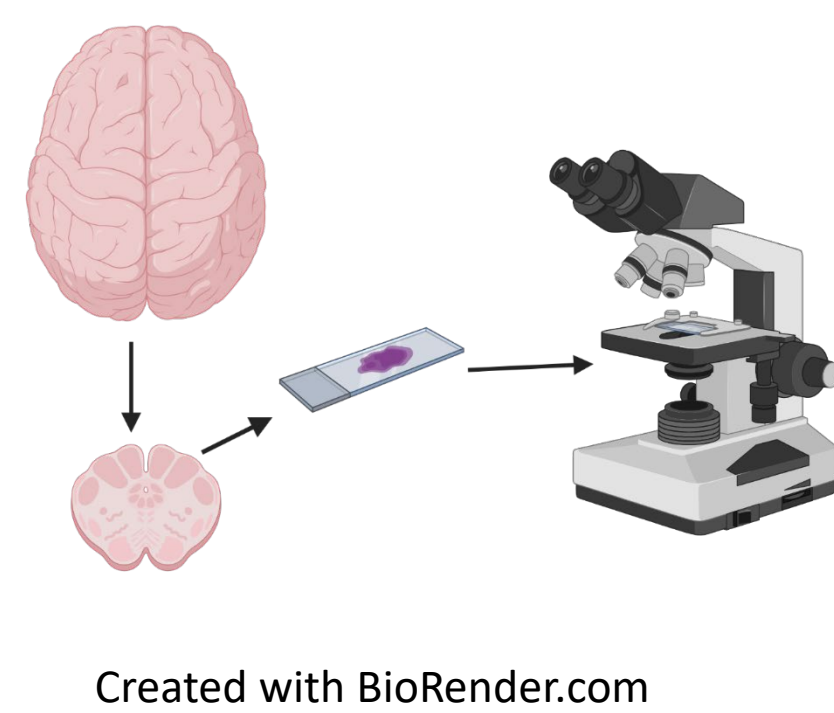
## Conclusions

Our study demonstrates for the first time the presence of a bastrovirus (PoBastV-CH22) in neurons and axons in the CNS of pigs, in combination with a severe non-suppurative meningoencephalomyelitis. Mainly the grey matter of the spinal cord and the brain was affected, showing neuronal satellitosis, neuronophagia, glial nodule formation and mainly lympho-plasmacellular perivascular cuffing and infiltration of the meninges. We determined the full-length genome of PoBastV-CH22 by HTS and RACE. Since this sequence clusters phylogenetically with other porcine bastroviruses from pigs without neurological signs, it is so far unknown, why neuroinvasion and disease occurred in the three examined pigs infected with PoBastV-CH22. It may be due to genetic differences of the viral strains and/or host-related or environmental factors. Considering the relationship to hepeviruses and astroviruses, one should be aware of bastrovirus infections especially in immunocompromised individuals with neurological signs.

## Materials and Methods

### A) Histopathological examination

- brain
- spinal cord
- dorsal root ganglia

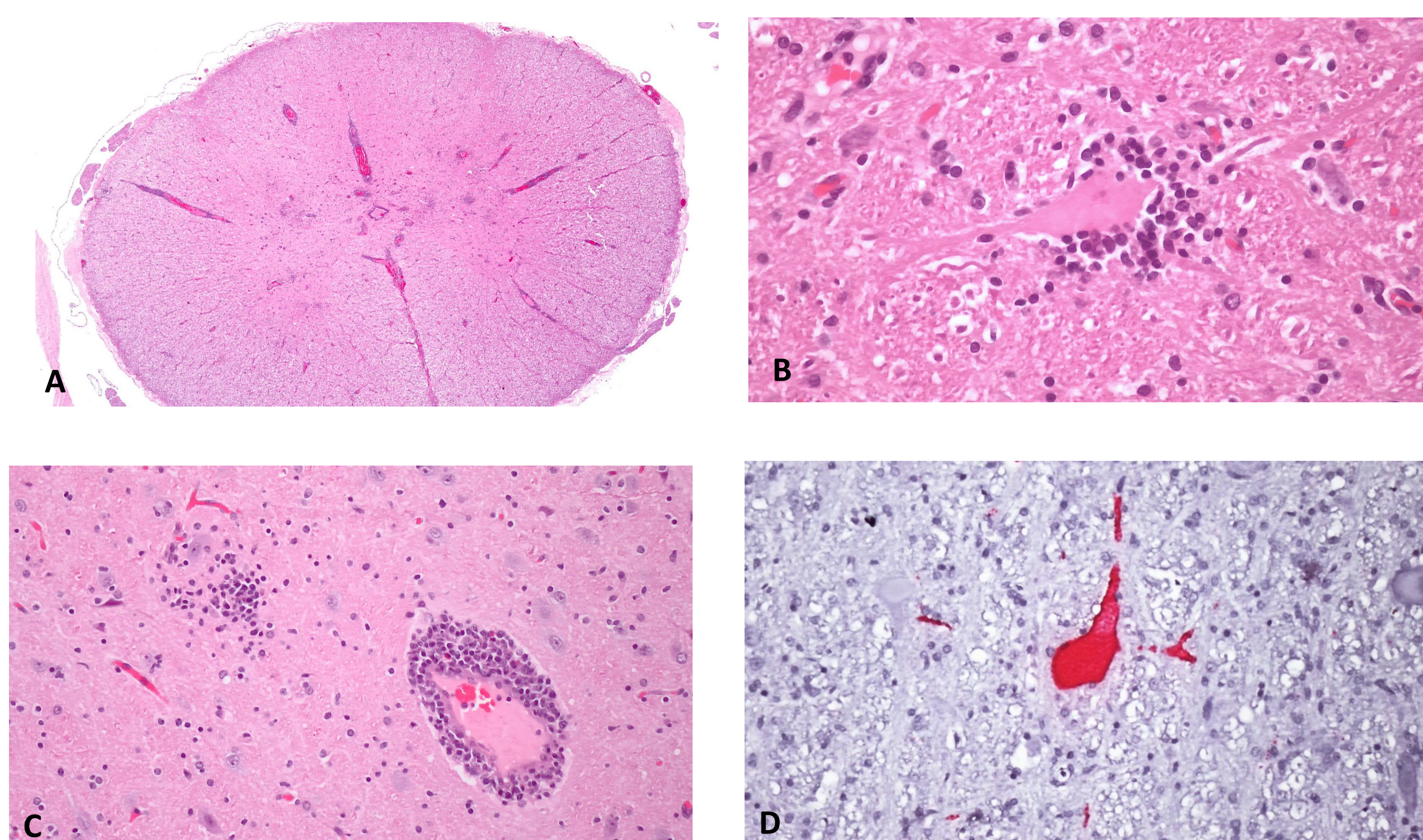


### B) Workflow

- RNA extraction from fresh brain
- High throughput sequencing (HTS)
- Bioinformatics-based virus discovery
- Probe design for (ISH) and primer design for RACE



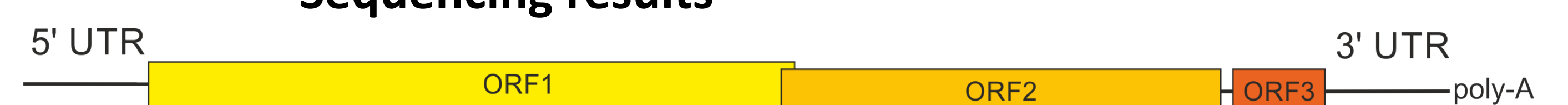
## Histopathological lesions and ISH staining



**Figure 1:** **A)** Overview of spinal cord showing a non-suppurative meningomyelitis, HE 2x; **B)** Medulla oblongata with neuronal satellitosis and neuronophagia, HE 40x; **C)** Thalamus with lympho-plasmacellular perivascular cuffing and glial nodule formation, HE 20x; **D)** Medulla oblongata with positive signal for PoBastV-CH22 RNA in neuronal body and axons, demonstrating the neurotropism of this virus, ISH 20x.

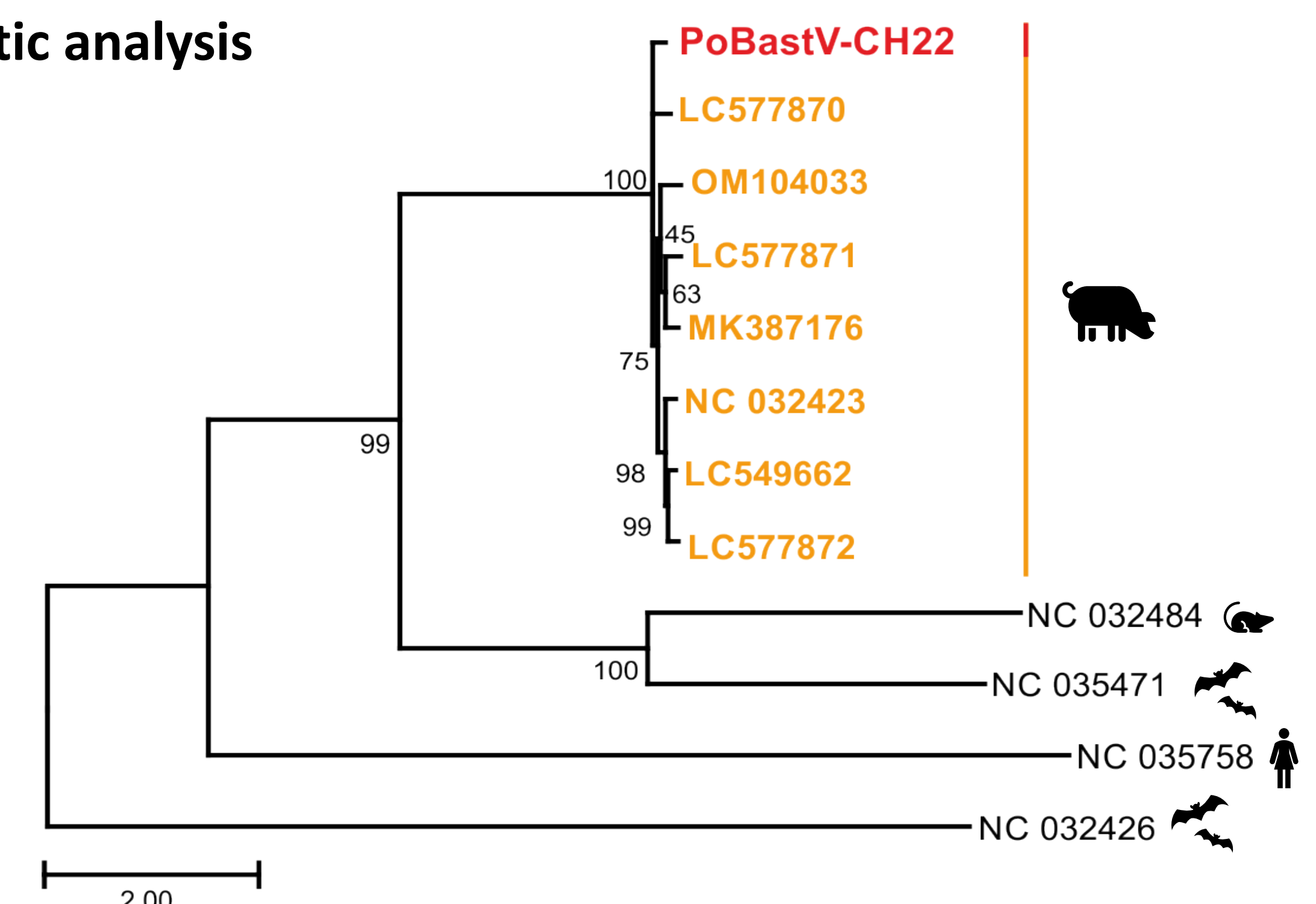
## Results

### Sequencing results

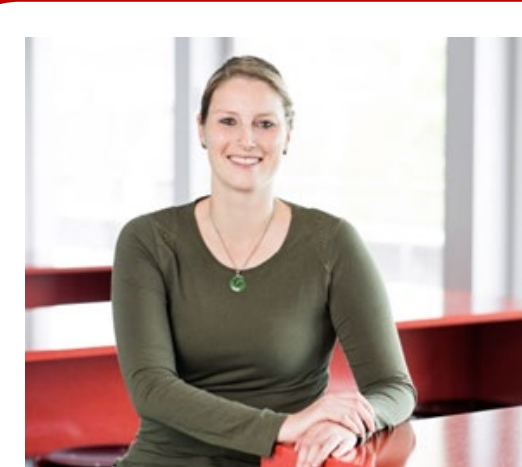


**Figure 2:** We obtained an almost full-length sequence of PoBastV-CH22 (kmer coverage 326). As in other bastroviruses, the open reading frame (ORF1) is similar to *Hepeviridae* and the ORF2 to *Astroviridae*<sup>1</sup>. Our strain has a third predicted ORF3, which is already described in human bastroviruses. The genome has a length of ca. 6 kilobases.

### Phylogenetic analysis



**Figure 3:** PoBastV-CH22; red clusters to the already known porcine bastroviruses (orange). The analysis was performed with porcine strains and reference sequences of human, rat, and bat strains. Strains are indicated with their GenBank Accession numbers. The alignment was done using the MAFFT (Ver. 7.475) software. A maximum-likelihood tree was constructed with IQ-Tree (Ver. 2.0.3) with 5'000 bootstrap replicates.



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### Literature

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- 2 Nagai M, Okabayashi T, Akagami M et al. Metagenomic identification, sequencing, and genome analysis of porcine hepe-astroviruses (bastroviruses) in porcine feces in Japan. *Infection, Genetics and Evolution* 2021; **88**: 104664.
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- 4 Reuter G, Pankovics P, Boros A. Nonsuppurative (Aseptic) Meningoencephalomyelitis Associated with Neurovirulent Astrovirus Infections in Humans and Animals. *Clinical Microbiology Reviews* 2018; **31**. doi:10.1128/CMR.00040-18