



Molecular Studies on Infections with Two Nidoviruses: Bovine Coronavirus and Equine Arteritis Virus

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Master of Science Programme for International Students
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To my parents

Abstract

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Infections with nidoviruses can cause various problems in animals and human beings. Bovine coronavirus (BCoV) has been considered as an important pathogen, causing severe diarrhea of newborn calves, winter dysentery of adult cattle, and respiratory disease in calves. Since this virus is repeatedly reported to cause severe disease in the Swedish cattle population, it is important to develop a highly sensitive diagnostic method for the detection of BCoV and to study the genetic characteristics of virus involved in the outbreaks.

A nested RT-PCR method targeting the HE gene was developed, which can be successfully applied for the diagnosis of BCoV infection. A conventional PCR was used to amplify the S gene of BCoV. Sequence analysis of the S gene showed a genetic diversity among Swedish and Danish virus isolates. S gene sequencing did not reveal differences between viruses from nasal and fecal swabs originating from the same animal, suggesting that the same virus can cause respiratory and enteric disease.

Horses are infected with equine arteritis virus (EAV) primarily through the respiratory and venereal routes. The outcome can vary greatly from subclinical infection to systemic disease characterised by fever, nasal discharge, lacrimation, arteritis, abortion, fetal death, and persistent infection in stallions. The LP3A1+ strain, a virus stock that was obtained by one additional cell culture passage of the LP3A virus, a large plaque variant of EAV Bucyrus strain, in equine embryonic cells was shown to be more virulent than the LP3A virus. An experimental infection has been done with a group of 14 horses to further characterise the *in vivo* biology of this LP3A1+ virus.

Analysis of open reading frames (ORFs) encoding EAV glycoproteins (GP) has shown the occurrence of mutations in the course of the experimental infection. While ORFs 2b and 3 had stabilizing mutations, ORFs 4 and 5 were subject to positive selection. Mutations in ORF5 mainly clustered in variable region 1, resulting in new genetic variants with changed neutralization domain in the GP5 (previously termed G_L). The new virus variants, however could not be entirely related to induction of recurrent viraemia in the infected horses, which is presumed to have been caused by other factors. It would be of interest to investigate the occurrence of other forms of persistent infection, rather than carrier stallions, upon infection with EAV.

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Comparative nucleotide sequence analysis of Bovine Coronavirus (BCoV) isolates from Sweden and Denmark
Lihong Liu, Mikhayil Hakhverdyan, Sara Hägglund,
Lars Erik Larsen, Stefan Alenius, and Sándor Belák

Research Report 2 **31**

Analysis of ORFs 2b, 3, 4, and partial ORF5 of sequential isolates of equine arteritis virus (EAV) shows genetic variation following experimental infection of horses
Lihong Liu, Javier Castillo-Olivares, Nick J. Davis-Poynter, Claudia Baule, and Sándor Belák

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