AHEAD IN
ANIMAL HEALTH

Phylogenetic analysis of BoHV-1 from Dutch cattle in 1995-2020 reveals high genetic heterogeneity

Remco Dijkman, Jet Mars, Frederik Waldeck, Rianne Buter, Annelies Hoogkamer, Kees van Maanen, Paul Wever
Royal GD, Deventer, The Netherlands

Introduction

In several European countries Bovine herpesvirus-1 (BoHV-1) monitoring and vaccination programmes are in place. Knowledge regarding the genetic variability and the phylogenetic relationship of virus strains is important in such programmes. In the Netherlands, testing nasal samples of cattle with respiratory symptoms is part of the monitoring programme. Yearly, nasal samples of approximately 100-300 cattle herds are admitted to Royal GD and tested by virus isolation and/or a real-time PCR.

Materials and Methods

- 33 nasal swabs and 172 virus isolations, from 1995-2020; including 12 BoHV-1 samples from Germany and 2 samples from India were submitted for whole genome sequencing on a Illumina Novaseq
- Reference mapping was performed using CLC Genomics Workbench 21.0.5. and BoHV-1.1 Strain Cooper (JX898220) as reference.

Results

- A median sequencing depth of 306X (range: 12X-4620X) was observed and median coverage was 98.6%.
- In total 1238 informative SNPs were used for construction of the SNP tree (Figure).
- All Dutch isolates belonged to type BoHV-1.1. One isolate obtained from Germany and two isolates from India were of the BoHV1.2 type.
- Over 52 genetically different 'clusters' could be distinguished using 1 SNP difference for cluster separation.
- BoHV-1 samples from different animals of the same farm always showed an identical sequence.
- For 28 samples both the nasal swab and the isolated virus sequences were identical.
- Spatiotemporal analysis revealed 36 samples that belonged to 9 different clusters with suspected epidemiological link (indicated in the figure (I – IX)).

Conclusion and Discussion

- Isolation, molecular characterization, and phylogenetic analysis of BoHV-1 strains provides valuable information to better understand the epidemiology of BoHV-1. In the end phase of a control programme and in cases of re-introduction in BoHV1 free regions, such data can be used for molecular epidemiologic analysis of outbreaks.
- Although primary samples (nasal swabs) are very suitable for diagnostics by real-time PCR, genotyping was most successful after virus isolation.
- Samples obtained from outbreaks with suspected epidemiological link often showed identical sequences or only 1 SNP difference. However, also samples from unrelated outbreaks (both in time and place) sometimes showed small numbers of SNP differences.
- Therefor good quality sequencing data, completeness of the genome and sufficient coverage are important in allowing reliable genotyping and molecular epidemics.

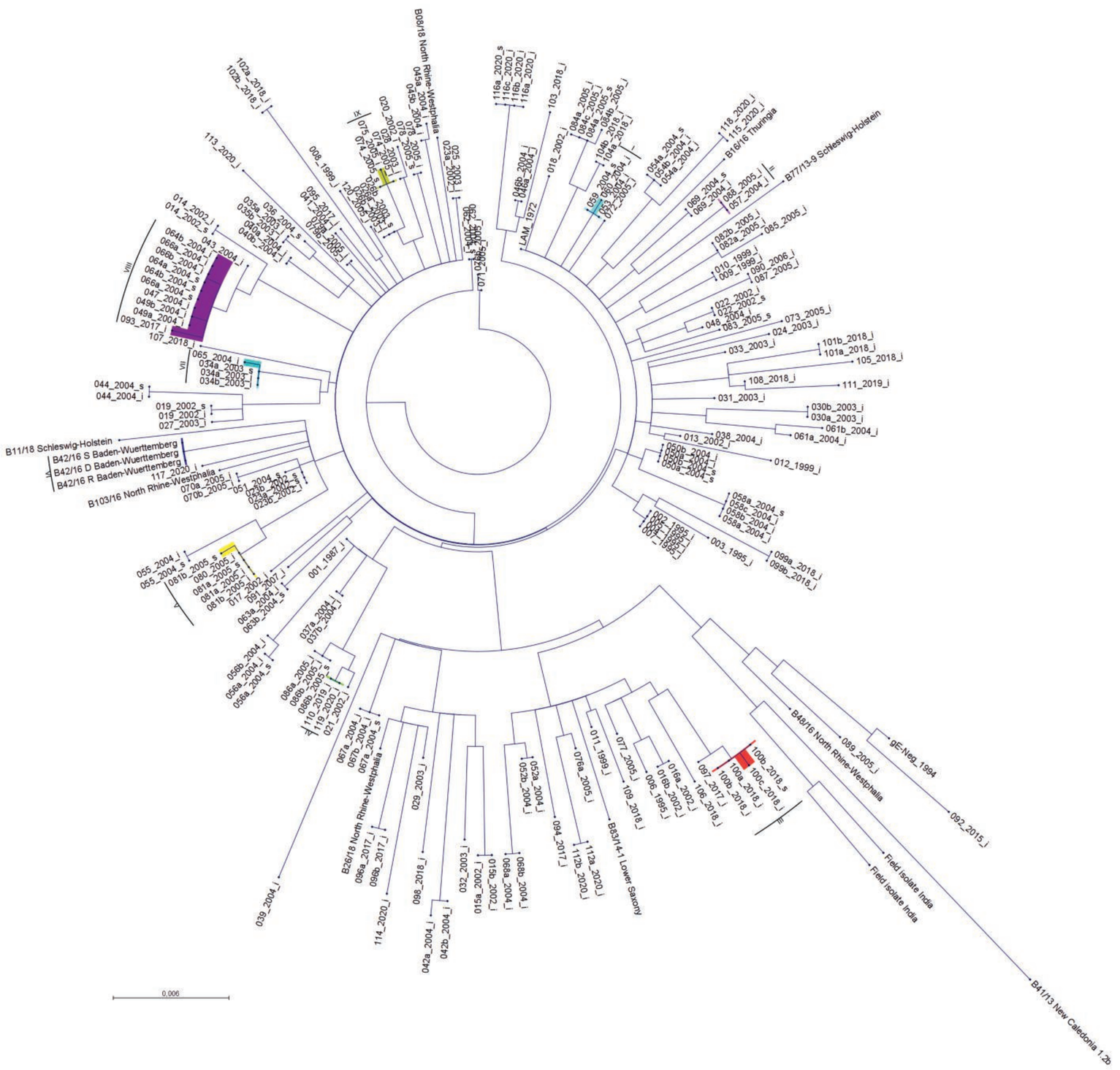

Figure 1. Circular unrooted phylogenetic SNP tree based on 1238 informative SNPs from 205 BoHV-1 samples and using BoHV-1.1 Strain Cooper (JX898220) as reference. Data were clustered using the neighbour joining method and the Jukes Cantor nucleotide substitution model. Samples originating from Dutch cattle are identified according to farm they originated from, year of isolation and sample type (s for nasal swab and i for virus isolation). Samples that where obtained from different animals on the same farm are indicated with a-c (e.g. 040a). Clusters of samples with suspected epidemiological link are indicated in different colors and number I-IX.

r.dijkman@gdanimalhealth.com
www.gdanimalhealth.com