Evolutionary analysis of whole-genome sequences confirms inter-farm transmission of Aleutian mink disease virus

Aleutian mink disease virus (AMDV) is a frequently encountered pathogen associated with mink farming. Previous phylogenetic analyses of AMDV have been based on shorter and more conserved parts of the genome, e.g. the partial NS1 gene. Such fragments are suitable for detection but are less useful for elucidating transmission pathways while sequencing entire viral genomes provides additional informative sites and often results in better-resolved phylogenies. We explore how whole-genome sequencing can benefit investigations of AMDV transmission by reconstructing the relationships between AMDV field samples from a Danish outbreak. We show that whole-genome phylogenies are much better resolved than those based on the partial NS1 gene sequences extracted from the same alignment. Well-resolved phylogenies contain more information about the underlying transmission trees and are useful for understanding the spread of a pathogen. In the main case investigated here, the transmission path suggested by the tree structure was supported by epidemiological data. The use of molecular clock models further improved tree resolution and provided time estimates for the viral ancestors consistent with the proposed direction of spread. It was however impossible to infer transmission pathways from the partial NS1 gene tree, since all samples from the case farms branched out from a single internal node. A sliding window analysis showed that there were no shorter genomic regions providing the same phylogenetic resolution as the entire genome. Altogether, these results suggest that phylogenetic analyses based on whole-genome sequencing taking into account sampling dates and epidemiological data is a promising set of tools for clarifying AMDV transmission.

General information
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Molecular diagnostics of aleutian mink disease virus: applied use of next generation sequencing and phylogenetics

Aleutian Mink Disease virus (AMDV) is a parvovirus causing Aleutian Mink Disease (AMD), often referred to as plasmacytosis. It is a systemic infection affecting mink of all ages, and is globally the most important pathogen impacting mink farming. In Denmark AMDV has since 1999 been monitored by a national control program, which is based on serological screening of all animals and encourages infected farms to stamp out. Historically there has been no consensus about which genomic region of the virus to analyse e.g. in relation to surveillance, and most previous studies in this regard, have been based either on partial or entire genes, or on pure epidemiological data. Thus, when initiating this project, little was known about AMDV’s total genomic diversity and how the virus was spread between farms.

Recent advances in the field of molecular diagnostics have made high throughput tools such as next generation sequencing cheaper and more easily available. Whole genome sequencing and advanced phylogenetic analyses have successfully been applied to describe the molecular evolution and transmission patterns for viruses such as Foot and Mouth Disease Virus (FMDV), Ebola, and avian influenza virus, however not previously for AMDV. The overall aim with this thesis was to investigate if next generation sequencing and phylogenetic analyses of full length isolates could improve our understanding of the total genomic diversity and evolution of AMDV. Additionally, we wanted to evaluate if this knowledge could contribute to the elucidation of AMDV transmission between farms and improve molecular diagnostics. During the first phase of this project a method for performing whole genome sequencing of AMDV was developed. This protocol enabled the sequencing of a large number of in vivo infectious AMDV isolates and provided the necessary dataset to act as foundation for the remaining analyses in the thesis. The first original paper (Manuscript 1) describes this protocol.

Manuscript 2 is a proof-of-concept study which demonstrated the advantage of using the whole genome sequence approach, compared to the in Denmark traditionally used partial NS1 gene sequencing, for the elucidation of transmission pathways between farms. The study was performed on samples from a small local AMDV outbreak, and clearly illustrated that the phylogenies based on partial NS1 gene sequencing were uninformative and could not be used for determining transmission pathways, even in the light of supporting epidemiological data. The whole-genome approach on the other hand, confirmed the epidemiological hypothesis about the direction of spread.

In Manuscript 3, the methodologies from Manuscript 1 and 2 were applied to generate the to-date most comprehensive phylogenetic and genetic analysis of full-length AMDV isolates, composed of more than 200 field strains. The study shed light on the diversity and evolutionary behaviour of two distinct AMDV strains, in addition to providing the first robust evolutionary rate-estimates. Altogether, the work presented in this thesis provides a contribution to the molecular diagnostics of AMDV, enables us better to understand the virus’ evolutionary behaviour in the context of mink farming, and is anticipated to be of value for more accurately tracing back in time the emergence of future outbreaks.
A fast and robust method for whole genome sequencing of the Aleutian Mink Disease Virus (AMDV) genome

Aleutian Mink Disease Virus (AMDV) is a frequently encountered pathogen associated with commercial mink breeding. AMDV infection leads to increased mortality and compromised animal health and welfare. Currently little is known about the molecular evolution of the virus, and the few existing studies have focused on limited regions of the viral genome. This paper describes a robust, reliable, and fast protocol for amplification of the full AMDV genome using long-range PCR. The method was used to generate next generation sequencing data for the non-virulent cell-culture adapted AMDV-G strain as well as for the virulent AMDV-Utah strain. Comparisons at nucleotide- and amino acid level showed that, in agreement with existing literature, the highest variability between the two virus strains was found in the left open reading frame, which encodes the non-structural (NS1–3) genes. This paper also reports a number of differences that potentially can be linked to virulence and host range. To the authors’ knowledge, this is the first study to apply next generation sequencing on the entire AMDV genome. The results from the study will facilitate the development of new diagnostic tools and can form the basis for more detailed molecular epidemiological analyses of the virus.
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Projects:

Identification of risk factors for acquiring ADV in Danish mink farms

Department of Bio and Health Informatics
Period: 01/08/2013 → 06/06/2017
Number of participants: 7
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Main Supervisor:
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Examiner:
Sicheritz-Pontén, Thomas (Intern)
Decaro, Nicola (Ekstern)
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**Bedre smittesporing med supercomputer**
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**Media contribution (1)**

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17/09/2016
Dynamo, Print
Julie Iben Schmidt
http://www.dtu.dk/Om-DTU/Nyheder-og-presse/Dynamo
Emma Elisabeth Hagberg
Molecular Evolution, Department of Bio and Health Informatics, Disease Intelligence and Molecular Evolution
Press / Media

**Computerome - Kopenhagen Fur**
Emma Elisabeth Hagberg
10/12/2015
Molecular Evolution, Department of Bio and Health Informatics, Disease Intelligence and Molecular Evolution

**Media contribution (1)**

**Computerome - Kopenhagen Fur**
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Youtube, Web
Julie Iben Schmidt
https://www.youtube.com/watch?v=HPsWZzi5Gkg
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Molecular Evolution, Department of Bio and Health Informatics, Disease Intelligence and Molecular Evolution
Press / Media