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Short Communication

Monitoring chronic infection with a field strain of Aleutian mink disease virus

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ABSTRACT

Aleutian mink disease virus (AMDV) readily spread within farmed mink and causes chronic infections with significant impacts for welfare and economy. In the present study a currently circulating Danish AMDV strain was used to induce chronic experimental infection of farmed mink.

PCR was used to detect viral DNA in full blood, organs, faeces and oro-nasal swabs weekly for the first 8 weeks and then biweekly for another 16 weeks after AMDV challenge inoculation of wild type mink. The mink ($n = 29$) was infected and seroconverted 2–3 weeks after AMDV inoculation and AMDV antibodies persisted during the maximum experimental period of 24 weeks. Viraemia and faecal excretion of viral DNA was detected in the mink ($n = 29$) at various and intermittent time intervals. Excretion of viral DNA in oro-nasal swabs was detected for 1–8 weeks in 21 mink. This highlights the risk of transmitting AMDV between infected farms.

PCR was successfully used to detect viral DNA in organs 8, 16 and 24 weeks after AMDV inoculation with only minor differences between these weeks which is of diagnostic interest.

This AMDV challenge model was also used to mimic natural infection of susceptible sapphire mink. Four of 6 sapphire mink were infected indirectly via the AMDV inoculated wild type mink whereas the other 2 sapphire mink remained uninfected.

1. Introduction

Aleutian mink disease virus (AMDV) is a parvovirus belonging to the genus *Amdovirus*. AMDV causes multiple clinical syndromes world-wide in farmed mink (reviewed by Bloom et al., 1994) and infect many wild mustelids (reviewed by Farid, 2013; Jensen et al., 2012). The outcome of AMDV infection in mink depends on the age of the mink and host factors such as genotype, virus strain is another important factor for development of Aleutian Disease (AD) (Alexandersen, 1990; Hadlow et al., 1983; Oie et al., 1996). Mink carrying the Aleutian gene e.g. sapphire and pastel mink, are the most susceptible for developing severe and rapidly progressive AD (Hadlow et al., 1983). The pathogenesis of AMDV strains circulating in the 1980'ies such as AMDV-Utah, AMDV-DK have been extensively studied using immunohistochemistry, southern blots and in situ hybridisation and immunoelectrophoresis (reviewed by Alexandersen, 1990; Bloom et al., 1994; Porter et al., 1980).

Recently, various PCR primers have been developed to detect AMDV DNA some amplifying capsid gene (VP2) (Jackson et al., 1996; Jahns et al., 2010; Oie et al., 1996) and others the non-structural protein (NS1) (Jensen et al., 2011; Knuuttila et al., 2009; Olofsson et al., 1999). However, the present study is the first systematically PCR analysis of organs during chronic AMDV infection

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including test of oro-nasal and faecal samples for excretion of viral DNA induced by a current AMDV field strain, Saeby/DEN/799.1/05. This AMDV field strain is the most prevalent in Denmark and significantly different from previous AMDV strains used in experimentally infections (Christensen et al., 2011). The aim of the present study was to monitor what happens in millions of mink acquiring this infection and thereby elucidate the pathogenesis of currently circulating AMDV with contemporary diagnostic methods.

2. Materials and methods

2.1. Mink

Mink (*Neovison vison*) were purchased from two neighbouring farms sharing breeding animals both farms were located on Zealand, which has been AMDV free island for more than a decade. The mink (42 wild type mink and 6 sapphire mink) were tested negative for antibodies against AMDV before they were moved to the experimental facility at the National Veterinary Institute (NVI), Technical University of Denmark. The farms had no records of previous AMDV, mink enteritis virus or other infectious diseases and no records of any vaccinations. The care and use of the animals was in accordance with the guidelines and approved by the Danish Animal Care and Ethics Committee.

At the animal experimental facility the 48 mink were housed indoor in standard mink cages in 4 isolation rooms designed for animal experiments with 12 mink cages in one row in each room. Each mink was housed individually in standard mink wire mesh cages with a wooden nest box. The mink did not have direct physical contact with each other, but indirect contact via for example saliva between the wire mesh cages could take place. The mink were fed a standard mink diet from a commercial mink feed producer. At arrival to the animal experimental facility all mink were clinically assessed and the mink had 2 days for acclimatisation before AMDV inoculation (day 0). One mink was euthanized before the AMDV inoculation for health reasons and thus not included in the data analysis.

2.2. Virus used for AMDV challenge inoculation

An organ homogenate was prepared by homogenisation of spleens from 7 mink with chronic AMDV infection, collected from one farm in the area of Northern Jutland where AMDV is endemic (Christensen et al., 2011). All spleens were positive for AMDV DNA by PCR and sequenced as described by Jensen et al., 2011. The AMDV sequences were identical to Saeby/DEN/799.1/05, the most prevalent strain of AMDV currently circulating in Denmark (Christensen et al., 2011). The homogenate (20%) was prepared in phosphate buffered saline (PBS) added 4% penicillin/streptomycin and then diluted 1:100, 1:1,000, 1:10,000 in PBS.

The homogenate was titrated 5-fold starting with an undiluted sample. Each of 8 dilutions was tested by PCR as described by Jensen et al. (2011). This titration was repeated 4 times to determine the highest detectable dilution of the homogenate which was 1:625. Based on titrations with AMDV-G a 1:10,000 dilution of challenge homogenate will comparably approximately contain 1.56×10^4 ng viral DNA/ml (Jensen et al., 2011). Each of the undiluted homogenate samples was sequenced with the PCR primers of NS1 gene and VP2 genes (Saifuddin and Fox, 1996). The homogenate was tested for absence of bacteria on blood agar (Blood agar base No. 2, Oxoid, Greve, Denmark) supplemented with 5% calf blood and incubated for 37 °C for two days. Further, the homogenate was tested for mink enteritis virus by ELISA (Uttenthal et al., 1999).

Finally, the challenge homogenate was titrated in vivo in mink, because field strains of AMDV cannot be cultured in vitro (Bloom et al., 1980). The aim of this titration was to find the lowest AMDV concentration causing a chronic subclinical infection. In total 12 one-year old female wild type mink were divided into 4 groups of each 3 mink given undiluted, 1:100, 1:1000 and 1:10,000 AMDV challenge homogenate diluted in PBS with 4% penicillin/streptomycin. The mink were anesthetised with ketamine/xylazine for AMDV inoculation and in the end before euthanasia with pentobarbital.

2.3. Chronic experimental infection with AMDV

The chronic infection experiment was initiated by inoculation of 29 wild type female mink with 1 ml of 1:10,000 dilution of the AMDV challenge homogenate (Fig. 1). Half of the mink were young (5 months old) and the other half of the mink were adult (17 months old). The mink were inoculated ip under anaesthesia with ketamine/xylazine. Use of ip inoculation would ensure information on the exact date of infection and dose of virus. In addition, 6 sapphire female mink, 3 mink of 5 months age and 3 mink of 17 months old, were kept as non-inoculated sentinels to mimic natural transmission of the infection (Fig. 1).

Every day each mink were clinically examined for animal welfare reasons and if mink showed clinically symptoms, they were euthanized ($n = 3$). Blood samples (non-stabilised and stabilised with EDTA) were taken weekly. Additionally, swabs were sampled from the oro-nasal region and faecal samples, probably urine contaminated, were collected weekly under the cage. Eight weeks after the AMDV inoculation 12 mink (5 young wild type females, 5 adult wild type females, 1 young sapphire female mink and 1 adult sapphire female mink) were euthanized with pentobarbital after anaesthesia with ketamine/xylazine (Fig. 1). After the first 8 weeks the blood, swab and faecal sampling were reduced to biweekly, but clinical assessments continued on a daily basis. The remaining two groups of 12 mink were euthanized 16 and 24 weeks after AMDV inoculation (Fig. 1). All mink were necropsied and lung, liver, spleen, mesenteric lymph node, kidney, bone marrow and brain was sampled and stored at −80 °C.

2.4. AMDV PCR analysis of organs, blood, swabs and faeces

Total DNA was isolated from mink organs, blood samples stabilised with EDTA and swab samples in PBS