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Inactivation of Aleutian mink disease virus through high temperature exposure *in vitro* and under field-based composting conditions

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ABSTRACT

Disposal of manure contaminated with Aleutian mink disease virus (AMDV) is a significant concern to the mink industry. Inactivation of AMDV under field conditions has received limited attention in the scientific literature. We evaluated the thermal inactivation of AMDV *in vitro* and during composting of mink manure. Spleen homogenate containing AMDV was heated under controlled conditions at 45 °C, 55 °C, and 65 °C for 3 days. Results of the *in vitro* study identified complete absence of viral replication in mink at 65 °C only. Next, manure-mixed AMDV packed in polyester pouches was inserted in different layers of three replicate mink manure compost piles. The virus was retrieved after the compost piles had undergone a heating period and subsequently returned to ambient temperatures. Temperature regimes in the compost piles were categorized as ≥65 °C, ≥60–64 °C, and ≥55–59 °C. Initially, layer-wise composite virus samples were assayed for virus replication in mink. Twenty-one-day post-inoculation (p.i.) plasma tested for AMDV and antibodies indicated infection in 40%, 80%, and 100% of mink inoculated from samples originating from the top, center and bottom layers of the piles, respectively. Subsequently, the virus was extracted from individual pouches in compost layers achieving thermal activity ≥65 °C and was tested in mink. No antibodies or virus was detected in plasma taken weekly up to day 21 p.i. PCR data of bone marrow and lymph nodes collected on day 21 p.i. also showed no AMDV. However, mink that received virus from positive control manure indicated infection in their plasma as early as 1 week p.i.

1. Introduction

Aleutian mink disease virus (AMDV, Carnivore amdoparvovirus 1), a member of the genus *Amdoparvovirus*, family Parvoviridae (Fang et al., 2010; Cotmore et al., 2014), causes serious economic losses to the mink industry. AMDV infections are marked by reductions in litter size (Padgett et al., 1967; Hansen and Lund, 1988) and increased mortality in newborn kits (Alexandersen et al., 1994). There is no effective treatment for the disease, and vaccination attempts have either failed or afforded partial protection (Castelruiz et al., 2005). Control of the virus is based upon detection and removal of seropositive mink tested mainly by counter-immunoelectrophoresis (CIEP) (Cho and Greenfield, 1978). The infected mink shed AMDV in their feces and urine, contaminating the manure to become a source of infection for healthy mink (Kenyon et al., 1963; Gorham et al., 1964). Disposal of virus-contaminated manure is a great concern for mink farmers. The manure is usually stockpiled outdoors on cleared land;

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however, some farmers manage manure through composting (Marel et al., 2008).

Various temperature regimes have been evaluated in the literature for inactivation of parvoviruses, which typically exhibit a high degree of resistance to disinfectants and heat (Eterpi et al., 2009). For example, heating of canine parvovirus (CPV) at 56 °C for 1 h and 7 h caused 0 and 53.3% reduction in infectivity, respectively, with 0.36% infectivity retention even after 72 h of treatment (Mcgavin, 1987). Moreover, exposure of CPV to 80 °C for 1 h resulted in a 96% reduction in infectivity. Boiling CPV at 100 °C for 2 min completely inactivated the virus when tested in cell culture (Mcgavin, 1987). Likewise, tissue preparations from AMDV-infected mink remained infectious after exposure to 80 °C for 30 min and 99.5 °C for 3 min (Gray, 1964). Comparable observations have been reported elsewhere in which AMDV was heated for 15 min at 90–95 °C with no effect on infectivity; however, a 30 min treatment at the same temperatures completely inactivated the virus (Burger et al., 1965). A study on the effect of heat for 30 min, found that the infectivity of a 650 nm Millipore membrane AMDV spleen filtrate was not compromised at 56 °C, nonetheless it was reduced by one and three $\log_{10}$ at 60 °C and 80 °C, respectively (Eklund et al., 1968).

Thermal conditions to inactivate microorganisms are not commonly found under normal environmental settings, and must be induced through management practices. Uttenthal et al. (1999) reported high stability of mink enteritis parvovirus over normal winter periods but under low moisture conditions viral inactivation was observed. Composting has been utilized to generate thermal conditions sufficient to reduce or eliminate viral pathogens to undetectable levels (Wichuk and McCartney, 2007). The impact of sawdust, straw and sawdust–straw base layer litter in conjunction with in-house poultry mortality compost was studied for Newcastle disease virus inactivation and it was observed that all litter materials maintained temperatures exceeding 60 °C over multiple days, however the virus was inactivated on day 2 of composting (Benson et al., 2008). The composting effect of poultry carcasses on the survival of highly pathogenic avian influenza virus and adenovirus of egg drop syndrome-76 showed that maximum temperature was 57.3 °C on day 4 of composting and that the viruses were inactivated at the end of the first 10 days of composting cycle (Senne et al., 1994). In another study, foot-and-mouth disease (FMD) virus-infected pig carcasses were exposed to temperatures between 50 and 70 °C within the initial 14 days in a chicken manure and wood shavings compost resulting in virus inactivation after 21 days (Guan et al., 2010). In the United States and Canada, a minimum compost temperature of 55 °C is required over 3–15 consecutive days to generate a Class A product with no detectable indicator pathogens (US EPA, 2003; CCME, 2005).

Most human RNA viruses, such as astroviruses, caliciviruses, enteroviruses, rotaviruses, and even some DNA viruses (adenoviruses), are readily destroyed at 55 °C in composted human feces (Guardabassi et al., 2003). One exception has been reported with the hepatitis-A virus, which requires 60 °C for 10 h for inactivation under composting conditions (Guardabassi et al., 2003). Studies of animal RNA viruses in manure slurries, including transmissible gastroenteritis virus and FMD virus, show no viral replication after exposure to temperatures as low as 50 °C for 1 h (Botner, 1990). Animal parvoviruses, on the other hand, exhibit a greater degree of heat resistance during composting. For instance, PPV requires 8 days for inactivation at 55 °C in a slurry (Botner, 1990), and in a laboratory-scale cattle manure compost model, complete inactivation of bovine enterovirus and bovine parvovirus (BPV) was observed at 60 °C when the viruses were sampled on day 28 of composting (Monteith et al., 1986). In contrast, using active compost consisting of stomach contents, fat, trims and blood of pigs (60%) and sawdust (40%), resulted in complete inactivation of BPV only after 7–9.5 days with peak temperatures reaching 57.2 °C for less than 5 h (Paluszak et al., 2010). Composting is an established method for pathogen reduction in animal manures and other organic by-products through generation of terminal thermal conditions. Mink manure compost has previously been reported to generate thermophilic conditions in excess of 60 °C for over a week (Ferguson, 2001), but no information is available on AMDV inactivation in compost. The present studies were undertaken to investigate thermal inactivation of AMDV both under lab-controlled conditions and through field-based mink manure composting.

2. Materials and methods

We undertook examining the effect of temperature exposure on AMDV infectivity using a series of laboratory and field experiments. We began by estimating AMDV ID_{50} and absorption kinetics, followed by an *in vitro* AMDV thermal exposure experiment with subsequent infectivity experiment using live animals. Upon completion of the laboratory experiments, a field composting study was conducted, using mink manure and other agricultural feedstocks, to establish temperatures similar to the laboratory experiment. Pouches with AMDV were inserted into the compost layers for exposure to the different temperatures and two live animal studies were subsequently completed to determine AMDV infectivity.

2.1. Animal handling

These experiments were carried out at the Aleutian Disease Research Center (ADRC), which houses AMDV-inoculated mink. AMDV-free black American mink (*Neovison vison*) obtained from two mink breeders in Nova Scotia, Canada, were used in these experiments. The mink were between 6 and 9 months old, including a mix of both genders, and were housed in a separate location at the facility to minimize the chance of cross-contamination. All protocols were approved by the institutional Animal Care and Use Committee according to the Canadian Council of Animal Care.