Understanding virus infectivity/viability in feed

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Take-Home Message

ASFV-like viruses can exist as a stable viable form, even in feed and feed constituents. Due to the ongoing outbreaks of ASF in Europe, Asia and most recently in the Caribbean Dominican Republic, we must now consider this third scenario (other than the reservoir or alternate host provided by soft ticks and wild pigs) of ASFV persisting stably in an altered but viable state in the environment, outside the highly susceptible domestic pig.

Abstract

African swine fever virus (ASFV) is a member of the family *Asfarviridae*, which is part of a larger group of virus families that are classified as nucleocytoplasmic large DNA viruses (NCLDVs). NCLDVs have evolved from a common ancestor, are found in a variety of environments, and can infect humans, fish, insects, swine, amoeba, and algae. Until now, no surrogate NCLDV with similar features to that of ASFV, nor any other virus with suitable surrogate properties, have been proposed for use in studies to evaluate virus survival and inactivation in feed matrices. Here, I will present data on an ecologically important NCLDV, Emiliania huxleyi virus strain 86 (EhV-86), which controls blooms of the marine unicellular phytoplankton *Emiliania huxleyi*. We found that ASFV and EhV-86 share many physical characteristics, such as complex virion ultrastructure and thermal stability. In fact, EhV-86 is one of the most thermally stable viruses, with temperatures up to 80°C damaging only the outer membrane of the virus, leaving the capsid and viral genome largely intact (confirmed by viability qPCR). The resultant thermally altered virion is now considered a viable but non-infectious particle (VNIP). Moreover, this VNIP is the dominant particle in a viable but non-infectious state (VNIS) that has the potential to be recovered, producing fully infectious viruses after multiple passages in its host, *E. huxleyi* (Balesteri et al., 2021).

Environmental stability appears to be a key factor in ASFV transmission. We hypothesize that VNIPs may play a role in the resistance of ASFV to heat-treatment. We speculate that higher temperatures may also render ASFV particles viable but non-infectious or viable but non-hemadsorbing. The ASFV thermal inactivation (as measured by hemadsorption (HAD_{50})) takes place only after lengthy exposure times (56 °C for 70 min or 60 °C for 20 min) (OIE, 2019). The HAD_{50} assay is reliant on the virus encoded CD2-like glycoprotein surface protein remaining intact in the envelope as this protein mediates hemadsorption (Borca et al. 2020). However, as shown for EhV-86, when ASFV virions are exposed to 60 °C, the capsid and enclosed genome of ASFV may remain intact when the surface glycoprotein layer is denatured. However, in doing so, the particle may still pose a danger as a VNIP because surface glycoproteins are not required for accidental phagocytosis, but results from the HAD_{50} assay will erroneously indicate a non-infectious sample. This observation further highlights the many challenges when applying ‘not fit for purpose’ analytical methods and consequent data misinterpretation of AFSV inactivation kinetics or survival data generated from various types of feed matrices (Shurson et al., 2021).

Given the similarities shared between ASFV and EhV-86, we proposed and used EhV-86 as a surrogate for ASFV in both *in vitro* and *in situ* feed inactivation experiments (Palowski et al 2021). We found that the NCLDV EhV-86 can be detected in a viable form collected from experimentally inoculated conventional and organic soybean meal, and complete feed based on corn and soybean meal, after a 23-d transcontinental truck transport journey. These results demonstrate for the first time that ASFV-like

NCLDVs can retain viability in swine feed matrices and can persist stably in an altered but viable state in the environment, outside the highly susceptible domestic pig.

References

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