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An update on current research in PRRS virus aerobiology

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Introduction

Transmission of swine viral pathogens by aerosols is frequently cited as a means of transmission within and between swine herds, but nearly all such claims are lacking the definitive evidence: detection of aerosolized virus.[2,10,13] In large part, the absence of corroborative data is due to technical challenges related to the collection of aerosolized virus, the detection of minute quantities of pathogen, and difficulties in differentiating between infectious and non-infectious aerosolized virus particles.

The objective of this paper is to briefly introduce current work in the area of PRRS virus aerobiology. Our overall objective is to understand the process of aerosol transmission. To do this, we have been working to obtain estimates of: (1) the quantity and duration of PRRS virus excreted by infected animals; (2) stability of aerosolized infectious virus in the environment; (3) the probability that a given dose will result in infection in a susceptible animal.

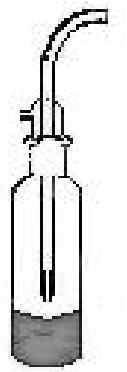
All of these variables require the ability to detect and quantify aerosolized PRRS virus - so this is the starting point.

Detection of PRRS virus in aerosols

The first technical problem is detection of PRRS virus in aerosols. Essentially, this is a diagnostic problem. In this case, the sample is air and the target is virus. There are air sampling methods reported in the literature, but there is little information on "diagnostic performance," i.e., the probability of detecting virus.

"Impingers" are samplers used to capture aerosolized viruses. Under vacuum, air is sucked into the impinger and impacted at a specific rate (liters/minute) on to a collection medium. "Impingement" results in the capture of airborne pathogens in liquid (**Figure 1**). Ideally, the quantity of virus (infectious and non-infectious) captured in the collection fluid should represent the density of virus (infectious and non-infectious) in the actual aerosol cloud. However, a number of variables affect the capture of viral particles in an air sampler: impinger type, sampling time, the composition of the sampling media, the concentration of aerosolized virus, and specific characteristics of the target virus.[8] Therefore, good "aerosol diagnostics" require the optimization and standardization of these variables.

Figure 1: Impinger. Air impacts the fluid at the bottom of the bottle and is captured in the liquid.



Optimization of aerosol collection media

Objective

We investigated various components used in collection media (antifoams, protectants, sorbents, ethylene glycol) with the objective of determining their effects on diagnostic assays for PRRS virus (virus isolation and PCR). Specifically, it was necessary to know whether these components affected the assays we use for detection of the virus, i.e., virus isolation and/or PCR.

Experiment

We investigated the effects of antifoams, protectants, sorbents, and ethylene glycol. Antifoams are used in collection media to prevent excessive foaming during air sampling (impingement). Protectants are used to prevent the inactivation of viruses via the physical disruption during impingement. Sorbents are materials used to adsorb viruses. The use of sorbents (activated carbon, kaolin, etc) has been described for improving the collection/detection of enteric viruses in contaminated water, but their use has not been investigated for air sampling.[1,15] We also investigated ethylene glycol with the objective of investigating the possibility of sampling at temperatures below 0° C.

Each component was evaluated independently for its effects on cell cultures, virus infectivity (PRRSV and SIV), PCR performance, and/or sampling efficiency.

Optimization of air sampling

Objective

The second step was to establish an optimized protocol for sampling PRRS virus in aerosols. That is, to determine which medium, impinger, and sampling time collected the most virus.

Experiment

A cloud of PRRS virus was generated using a nebulizer and held in a glass reservoir (**Figure 2**). This design made it possible to test and compare up to 6 different "sampling treatments" simultaneously. The collection efficiency of 3 impingers (AGI-30, AGI-4, SKC BioSampler(r)) and 6 different collection media was compared at multiple sampling points (0, 1, 2, 5, 10, 15, 20 minutes). The amount of PRRS virus in each sample was determined by quantitative RT-PCR to determine the optimal impinger, collection media, and sampling time.

Excretion from pigs

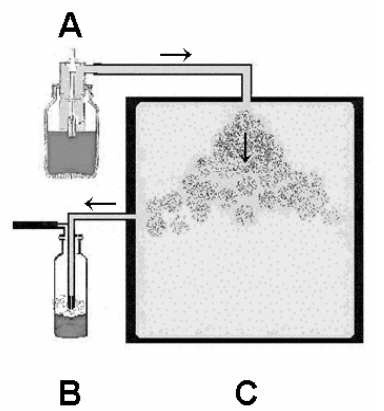
Objective

The third objective was to (1) determine the sensitivity of the impinger collection system (the least amount of virus detectable with the sampling system), then (2) quantify the virus excreted by individual pigs in exhaled air.

Experiment

Air samples were captured directly from pigs inoculated with one or more of the following: PRRS virus (ATCC VR-2332, MN-184, VR-2385), porcine circovirus type 2 (PCV-2), and *Mycoplasma hyopneumoniae*. Individual pigs were sampled by placing a canine anesthesia mask attached to the impinger collection system over the mouth and snout to collect exhaled air. Most pigs were sampled on post inoculation days 0, 1, 4, 6, 8, 11, 13, and 15. In addition to "air samples," oral fluid or nasal swabs were collected to document the presence of virus in the upper respiratory tract. Likewise, impinger samples of ambient air were collected and assayed for the presence of airborne environmental pathogens.

Figure 2: Design for optimizing collection. A. nebulizer B. impinger C. aerosol reservoir



PRRS virus stability

Objective

Aerosol transmission requires that the aerosolized virus remain infectious long enough to reach a susceptible pig. The rate at which inactivation occurs is one of the variables that determines the distance over which aerosol transmission can occur. Therefore, to understand aerosol transmission of PRRS virus, it is necessary to determine the rate of inactivation of aerosolized PRRS virus.

Experiment (in progress)

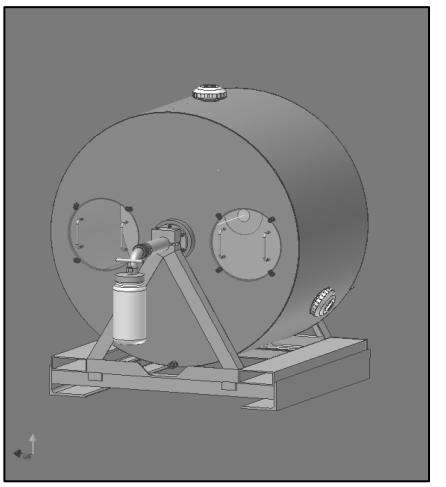
Environmental relative humidity and air temperature affect the stability and infectivity of aerosolized virus.[3,4,5,6,9,12,14,16,17] To test the effect of relative humidity and temperature, aerosolized PRRS virus is suspended in a rotating drum (dynamic aerosol toroid) (**Figure 3**) and sampled repeatedly over time. The rotation of the drum maintains the aerosol in suspension.[7] The rate of decay of PRRS virus (half-life) and total PRRS virus RNA will be determined over a range of temperatures and relative humidity.

Infectious dose by aerosol route

Objective

Determine the infectious dose for nursery pigs by aerosol route of exposure.

Figure 3: Dynamic aerosol toroid (DAT). Diagram created by Mike Harper, Iowa State University.



Experiment

An aerosol cloud will be generated and stabilized in the rotating drum describe above. Pigs will receive a specific dose of aerosolized PRRS virus (**Figure 4**). Accurate and precise estimates of exposure dose are critical in deriving valid dose-response curves.

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Figure 4: Infectious dose diagram. A. nebulizer B. impinger C. aerosol reservoir D. mask respiration system