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Short communication

A method to provide improved dose–response estimates for airborne pathogens in animals: An example using porcine reproductive and respiratory syndrome virus

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ABSTRACT

This paper describes a method to provide improved probability estimates that exposure to a specific dose of an airborne infectious pathogen will result in animal infection. Individual animals were exposed to a specific dose of airborne pathogen. Following exposure, animals were individually housed and monitored for evidence of infection. The detection of specific antibodies and/or the pathogen in diagnostic specimens was evidence that the exposure dose resulted in infection. If replicated over a range of doses, the results can be used to derive a dose–response curve for a variety of animal species and infectious pathogens. This information is useful in estimating the likelihood of infection associated with exposure to airborne infectious microorganisms. Applications include predicting the risk of transmission associated with exposure to airborne pathogens, modeling the transmission of airborne pathogens, and determining requirements for effective exposure doses for vaccines delivered in aerosols.

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1. Introduction

A wide variety of fungal, bacterial, and viral pathogens are transmissible in air (Tang et al., 2006). For humans, this includes re-emerging pathogens, e.g., *Mycobacterium tuberculosis* (Escombe et al., 2007), newly recognized pathogens, e.g., severe acute respiratory syndrome (SARS) coronavirus (Booth et al., 2005), and pathogens considered to have potential use as bioterrorism weapons (Atlas, 1998). For animals, this includes important zoonotic pathogens, e.g., avian influenza (Wong and Yuen, 2006), and economically significant pathogens of livestock, e.g., foot and mouth disease (Alexandersen et al., 2002).

A comprehensive understanding of airborne spread relies upon quantitative estimates of the steps in the transmission process: aerosolization of the pathogen (source, quantity, and duration); dissemination of the airborne pathogen (droplet size and air movement); retention of infectivity over time (relative humidity, temperature, ultraviolet radiation); and probability of infection as a function of exposure dose. Although an important route of disease transmission, our understanding of the process of airborne transmission is rudimentary for most infectious agents. In large part, this is due to technical challenges in achieving quantitative estimates of excretion, dissemination, stability, and probability of infection by exposure dose.

The objective of this study was to provide a method to achieve precise dose–response estimates for airborne pathogens. Porcine reproductive and respiratory syndrome virus (PRRSV) an enveloped RNA virus in the family *Arteriviridae* (Cavanagh, 1997) with a recognized potential for airborne transmission was used to validate the method.

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2. Materials and methods

2.1. Experimental design

The study was conducted as an incomplete randomized block design where pigs were exposed to a dose of airborne PRRSV. Seven replicates composed of 10 pigs per replicate were conducted. Within each replicate, 9 pigs were exposed to specific doses of airborne PRRSV. One pig served as an environmental sentinel, i.e., was in the room throughout the time treatments were administered, but was not intentionally exposed to PRRSV. Following treatment, pigs were housed individually to preclude transmission among animals. Serum samples collected at 0, 5, and 10 days post inoculation were assayed for the presence of PRRSV by virus isolation (VI) and reverse transcription-polymerase chain reaction (RT-PCR) to determine whether the exposure resulted in infection. The proportion of pigs infected at each exposure dose was used to derive the dose–response curve.

2.2. Source of airborne pathogen

A stainless steel DAT (Fig. 1) was constructed measuring 36 in. (91.4 cm) in diameter × 24 in. (60.9 cm) in depth (total volume of 400 l). The DAT was rotated at 4 RPM using a variable speed motor (BHLW15L-120T-D2, Brother il Gearmotors, Bridgewater, NJ) and housed within a custom-built refrigeration unit with the capacity to maintain temperatures between 4 and −20 °C (Carroll Coolers, Inc., 20590, Carroll, IA) (Fig. 1). The DAT was equilibrated at −19 °C, a temperature at which infectious PRRSV is highly stable (Hermann et al., 2007), prior to aerosolization of the virus. To allow for pressure equilibration during introduction of air (nebulization) and extraction of air (impingement and pig exposure), three hepa-filters (Fisher Scientific, 18-999-2574, Hampton, NH) were fixed to ports on the circumference of the DAT. Between replicates, the entire system was disassembled for cleaning and disinfection.

Isolate ATCC VR-2332 (American Type Culture Collection, Manassas, VA, USA; GenBank accession number PRU87392; Nelsen et al., 1999) was propagated in MARC-145 cells, as described in Hermann et al. (2007). To prepare one lot of high-titered PRRSV sufficient to conduct all the replicates, the propagated virus was pooled and concentrated using a Prep/Scale spiral wound ultrafiltration module (Model CDUF 001 LH, Millipore, Billerica, MA, USA) such that approximately 2 l of supernatant was reduced to approximately 200 ml of virus stock. The pooled virus stock was aliquoted into 10 ml volumes and stored at −80 °C.

For aerosolization, a suspension fluid was prepared: 90 ml of PBS ((1×) (Invitrogen, 10010-064, Carlsbad, CA, USA), 10 ml of PRRSV stock solution ($1 \times 10^{6.3}$ $TCID_{50}$/ml), 0.1% (v/v) Rhodamine B dye (Sigma Chemical Co., R6626, St. Louis, MO), and 0.01% (v/v) Antifoam A Emulsion (Sigma Chemical Co., A5758, St. Louis, MO)). The suspension fluid was aerosolized into the DAT using two 24-jet Collison nebulizers (BGI Inc., CN60, Waltham, MA) operating simultaneously for 10 min on compressed air (Sears Roebuck, 00916734000, Hoffman Estates, IL) at 40 P.S.I. These parameters produced 80 liters of free air per minute, a liquid generation rate of 1.1 ml per minute, and a particle size of 1.9 μm (May, 1973) (Fig. 1, Step 1).

2.3. Quantitation of airborne pathogen

SKC BioSampler® (SKC Inc., 225-9595, Eighty Four, PA) impingers were used to collect air samples from the DAT (Fig. 1, Step 2). SKC BioSampler® impingers were selected on the basis of comparisons of PRRSV collection performance among various samplers (Hermann et al., 2006). Each impinger contained 20 ml of sterile PBS (1×) collection fluid and was operated for 2 min. Impingers were operated to ensure a constant flow rate of 12.5 l per minute (l/min). Flow rate was verified using a flow meter (Dwyer Instruments Inc., DW-806, Michigan City, IN). Vacuum pressure was maintained using oil-less pumps (Fisher Scientific, S413801, Hampton, NH) and was monitored using a vacuum pressure gauge (Cato Western Inc., G-S4LM20-VAC-100, Tucson, AZ).

After nebulization, the aerosolized particles within the DAT were allowed to stabilize for 5 min before baseline air samples were taken. Thereafter, samples were collected immediately before and immediately after exposure of each pig to air within the DAT. After sampling, the collection fluid was assayed by: (1) PRRSV microinfectivity assay ($TCID_{50}$) to determine the titer of infectious virus per ml as described in Hermann et al. (2007); (2) PRRSV quantitative (qRT-PCR) to determine total genomic copies per ml as described in Wasilk et al. (2004).

2.4. Animal exposure to pathogen

Pigs were individually removed from isolation units on day 0, bled, and anesthetized using a combination of Telazol (5 mg; Fort Dodge Animal Health, Fort Dodge, Iowa), xylazine (250mg), and ketamine (250 mg; Fort Dodge Animal Health, Fort Dodge, Iowa). The xylazine and ketamine were used to reconstitute the lyophilized Telazol. The combination was administered intramuscularly at a dose of 1 ml per 22.5 kg of body weight. For exposure to the virus, the entire anterior portion of the head, i.e., snout and mouth, of the anesthetized pig was fitted with a canine surgical mask (SurgiVet, 32393B1, Waukasha, WI) attached with tubing (Fisher Scientific, 295736, Hampton, NH) to a one-way valve (Instrumentation Industries, Inc., BE-117, Bethel Park, PA) inserted into the DAT containing aerosolized infectious PRRSV (Fig. 1 Step 3). The cumulative volume (liters) of air inhaled by the animal during the exposure period was measured using a pediatric spirometer (Boehringer, 8805, Norristown, PA). In total, each pig was exposed to 10 l of virus-laden air from the DAT.

Impinger samples were collected immediately before and after each pig exposure to determine average titer of PRRSV per liter of air during the exposure period. The average concentration of infectious pathogen per liter of air (pre- and post-exposure) was determined and multiplied by the volume of air respired (10 l). The virus dose

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