EVALUATION OF A PROTOTYPE MECHANICALLY VENTILATED SWINE TRANSPORT TRAILER FITTED WITH AIR FILTRATION SYSTEM

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By

JINGJING PARAGSA CABAHUG

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ABSTRACT

In response to industry demand for a livestock vehicle that addresses both enhanced biosecurity and animal welfare during transport, a prototype air-filtered swine trailer was assembled. The prototype featured a swine trailer with two separate compartments: a front compartment that houses generator set, a bank of six air filter sets, ventilation controller, and two axial fans; and, the animal compartment with solid aluminum walls, two decks with hinged upper deck floor and a roof that can be lifted open, and a hydraulic lift gate which also served as the rear door. Two air inlet openings were installed on both sides of the front compartment while exhaust openings were on the side at the rear end of the livestock container. The goals of this current study were to evaluate the performance of the developed prototype trailer, conduct an economic analysis, and subsequently formulate recommendations for further optimization of the trailer design. Thus, in a stationary test, evaluation of the efficiency of the installed air filtration system (MERV 8 panel pre-filter and MERV 16 glass fiber V-bank filter) was carried out with no pigs inside the trailer. Upstream and downstream monitoring of concentrations of aerosolized model virus (bacteriophage Phi X174) yielded an overall filtration efficiency of 96.9%. Moreover, two monitoring trips with market-sized pigs loaded in the trailer showed a general front to rear movement of air as evidenced by increasing trailer temperature, moisture, and CO_2 levels from front to rear end of the livestock container. Conditions at the middle to rear portion of the animal compartment were maintained within acceptable thermal limits. However, locations close to the ventilation fans (front end of livestock compartment) experienced low temperatures (<10°C) during portions of the trip. Finally, cost analysis for a hypothetical 120-pig capacity air-filtered trailer yielded an estimated total equipment and installation cost of $109,900 and annual operational and filter maintenance costs of $9,520 and $600, respectively. Assuming an incremental revenue of $5 per head for biosecure pigs transported in an air-filtered trailer led to an estimated payback period of about 2.41 years for the trailer.

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TABLE OF CONTENTS

PERMISSION TO USE STATEMENT i

ABSTRACT……ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS iv

LIST OF TABLES viii

LIST OF FIGURES ix

NOMENCLATURE xi

CHAPTER 1 INTRODUCTION 1

CHAPTER 2 LITERATURE REVIEW AND RESEARCH OBJECTIVES 3

2.1 Airborne transmissible swine diseases and their economic impact 3

2.2 Airborne transmission of swine pathogens 5

2.2.1 Size distribution, concentration, viability in the environment and transport of pathogen-laden particles 5

2.2.2 Surrogate organisms in bioaerosol studies 11

2.3 Application of air filtration in the swine industry 13

2.3.1 Biosecurity in the swine industry 13

2.3.2 Air filtration systems adopted in swine barns 13

2.3.3 Financial implications of air filtration in swine barns 16

2.4 Existing swine transport scenario 17

2.4.1 Animal welfare concerns during transport 17

2.4.2 Existing swine trailers: Design and problems encountered 18

2.4.3 Mechanical ventilation in livestock trailers 20

2.4.3.1 Ventilation system effectiveness: Assessment method 20

2.4.3.2 Recent developments on mechanically ventilated livestock vehicles 21
2.5 Development of design for prototype air-filtered trailer 23
2.5.1 Conceptualization of initial design 23
2.5.2 Computer simulation and selection of final design 23
2.6 Summary 25
2.7 Knowledge gap 26
2.8 Research objectives 27
CHAPTER 3 MATERIALS AND METHODS 28
3.1 Overview of the research plan 28
3.2 Description of the prototype air-filtered trailer 28
3.2.1 Front compartment 29
3.2.2 Animal compartment 30
3.3 Stationary test 32
3.3.1 Preparation of the test virus 32
3.3.2 Aerosol generation and viral load sampling 32
3.3.3 Extraction of viral particles from filters and qPCR 35
3.3.4 Quantification of phage particles by qPCR 36
3.3.5 Calculation of filtration efficiency 37
3.4 Road test 37
3.4.1 Data collection 38
3.4.1.1 Temperature and humidity 40
3.4.1.2 CO_2, air speed and room static pressure 40
3.4.1.3 NH_3 and H_2S 41
3.4.2 Ventilation effectiveness 41

3.5 Data processing and analysis 41
3.5.1 Data processing 41
3.5.1.1 Viral concentration from qPCR 41
3.5.1.2 Environmental data from monitoring trips 42
3.5.2 Data analysis 43
3.6 Cost analysis and development of design optimization recommendations 43
CHAPTER 4 RESULTS AND DISCUSSION 44
4.1 Filtration efficiency test (stationary test) 44
4.1.1 Evaluation of the sampling set-up 44
4.1.2 Bacterial virus concentrations and filtration efficiency 46
4.2. Environmental condition inside the air-filtered trailer during pig transport (road test) 48
4.2.1 Details of the loads of pigs for the monitoring trips 49
4.2.2 Operation of the trailer mechanical ventilation system 50
4.2.3 Temperature distribution and overall thermal conditions during transport 52
4.2.3.1 Temperature time series for the entire transport 52
4.2.3.2 Temperature during stable transport period 55
4.2.3.3 Thermal comfort classification 58
4.2.4 Moisture levels and distribution 59
4.2.5 Carbon dioxide levels and distribution 62
4.2.6 Air speed 64
4.2.7 Effect of travel interruptions on trailer thermal condition and air quality 66
4.2.8 Ventilation effectiveness 68
4.3 Cost analysis and design optimization 70
4.3.1 Cost analysis 70

4.3.2 Recommendations for design optimization..... 73

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS 76

REFERENCES……..78

APPENDIX……90

LIST OF TABLES

Table 2.1. Common swine diseases than can be potentially spread airborne. 7

Table 2.2. Common viral aerosol models. 11

Table 3.1. Technical information on the filter media used. 29

Table 4.1. Filtration efficiency determined from reduction of bacteriophage Phi X174 concentration downstream of the air filters tested, n = 4. 45

Table 4.2. Summary of details on the loads of pigs used and the event durations for the two monitoring trips. 49

Table 4.3. Ventilation system control program applied for the prototype air-filtered trailer during the monitoring trips. 50

Table 4.4. Inlet and outlet temperatures (mean ± SD) in °C in the top and bottom decks of the prototype trailer during the stable transport period of the two monitoring trips..... 57

Table 4.5. Heat removal effectiveness (HRE) at different locations in the top and bottom decks of the animal compartment during the two monitoring trips..... 69

Table 4.6. Average contaminant removal effectiveness (CRE) at the center of the front and rear compartments of the top and bottom decks of the animal compartment during the two monitoring trips, n = 2. 69

Table 4.7. Costs associated with the assembly and operation of an air filtered trailer. 71

Table 4.8. Filter replacement cost. 72

Table A.1. Mean, minimum, maximum and standard deviation of the temperature and humidity ratio at various locations inside the trailer during the two monitoring trips……..91

LIST OF FIGURES

Figure 2.1. Pot belly (top) and straight deck (bottom) livestock trailers commonly found in Canada. 19

Figure 2.2. Screenshot of the geometry model of the air-filtered trailer. 24

Figure 2.3. Contours of predicted temperature inside the trailer at three different summer (A) and winter (B) outdoor temperatures from the sensitivity analysis. 25

Figure 3.1. Overall framework of the study. 28

Figure 3.2. Schematic diagram of the set-up of components of the trailer air filtration and ventilation system in the front compartment. 30

Figure 3.3. Photos of the animal compartment showing (A) its lower and upper decks, (B) hinged roof, (C) gate that partitions one deck into two pens, (D) air exhaust damper, (E) hydraulic lift gate, (F) hydraulic lift controller and (G) compartment exterior. 31

Figure 3.4. Diagram of the testing setup during the stationary test. 33

Figure 3.5. Photos of the testing setup. 34

Figure 3.6. Trailer schematic diagram showing the plan view of locations of sensors and data loggers used to measure temperature, relative humidity, air velocity, CO_2 and other gases (NH_3 and H_2S) levels inside the trailer. 38

Figure 3.7. Sensors and standalone data loggers used during the monitoring trips. 39

Figure 4.1. Total bacterial virus Phi X174 (in genome copies/m^3 of air) detected by qPCR. 47

Figure 4.2. Actual ventilation schedule for the two monitoring trips. 51

Figure 4.3. Variation in inlet, exhaust and internal trailer temperature measured at different locations approximately 1 m (≈ 40 in) above the floor along the center of the trailer top and bottom decks during the two monitoring trips. 53

Figure 4.4. Average air temperature during the stable transport period at different monitoring locations approximately 1 m (≈ 40 in) above the floor of the (A) top deck and (B) bottom deck of the animal compartment monitored during two monitoring trips, n = 2. 56

Figure 4.5. Assessment of overall trailer thermal condition by classification of observed temperatures at monitoring locations along the center of the trailer into thermal comfort categories. 59

Figure 4.6. Variation in inlet, exhaust and internal trailer humidity ratio measured at different locations approximately 1 m (≈ 40 in) above the floor along the center line of the trailer top and bottom decks during the two monitoring trips. 60

Figure 4.7. Average humidity ratio during the stable transport period measured in different locations approximately 1 m (≈ 40 in) above the floor along the center of the trailer top and bottom decks during the two monitoring trips, n = 2. 62

Figure 4.8. Variation in inlet, exhaust and interior CO_2 levels for both the trailer top and bottom decks during the two monitoring trips. 63

Figure 4.9. Average inlet, exhaust and interior CO_2 levels during transport (most environmentally stable period of the two monitoring trips) for both the trailer top and bottom decks during the two monitoring trips, n = 2. 64

Figure 4.10. Variation in air speed inside the trailer measured at the center of the front and rear compartments of the trailer top and bottom decks during the two monitoring trips.. 65

Figure 4.11. Average interior air speed during the stable transport period for both the trailer top and bottom decks during the two monitoring trips, n = 2. 66

Figure 4.12. Change in (A) temperature, (B) humidity ratio and (C) CO_2 levels before and during a short stop (7:12 - 7:22 am) during the December 1, 2017 monitoring trip. 68

Figure 4.13. Sensitivity analysis of air-filtered trailer investment payback period considering a range of premiums (in $/yr) for every genetic stock transported. 73

Figure A.1. Screenshot of the route taken during the two monitoring trips.....90

Figure A.2. Equivalent volume flow rates in cfm of the top and bottom deck fans at different % fan capacities……...…..……..90

NOMENCLATURE

Abbreviations	
ADV	Aujeszky's disease virus
AOZ	Animal occupied zone
ASHRAE	American Society of Heating, Refrigerating and Air-Conditioning Engineers Inc
ATCC	American Type Culture Collection
CFIA	Canadian Food Inspection Agency
CFD	Computational fluid dynamics
CO_2	Carbon dioxide
Cq	Quantitation cycle
CRE	Contaminant removal effectiveness
CSFV	Classical swine fever virus
DIP	Dead in pen
DIY	Dead in yard
DNA	Deoxyribonucleic acid
DNQ	Detected not quantifiable in qPCR
DOD	Dead or down on arrival
DOP	Dioctyl phthalate
DQ	Detected and quantified in qPCR
dsDNA	Double-stranded DNA
dsRNA	Double-stranded
EU	European Union
FMDV	Foot and mouth disease virus
H_2S	Hydrogen sulfide

HEPA	High efficiency particulate arrestance
HRE	Heat removal effectiveness
IAV	Influenza A virus
LSD	Least significant difference
MERV	Minimum efficiency reporting value
M hyo	*Mycoplasma hyopneumoniae*
MID	Minimum infectious dose
ND	Not detected in qPCR
NH_3	Ammonia
PEDV	Porcine epidemic diarrhea virus
PRDC	Porcine respiratory disease complex
PRRSV	Porcine reproductive and respiratory syndrome virus
RH	Relative humidity
RNA	Ribonucleic acid
SIV	Swine influenza virus
SPSS	Statistical Package for Social Sciences
SQ	Starting quantity
ssDNA	Single-stranded DNA
ssRNA	Single-stranded RNA
qPCR	Quantitative polymerase chain reaction
TMHP	Total metabolic heat production
TQA	Transport Quality Assurance
UV	Ultraviolet
VEF	Ventilation effectiveness factor
VFD	Variable frequency drive

VFR	Ventilation flow rate
WCVM	Western College of Veterinary Medicine
WHO	World Health Organization

Symbols

C	Viral or phage concentration of the sample, ssDNA copies/mL
C_{inlet}	Inlet CO_2 concentration, ppm
C_{outlet}	Outlet CO_2 concentration, ppm
C_p	Volumetric heat capacity of air, $J/m^3/°C$
f	Sampling flow rate, L/min
L_d	Average downstream viral load, ssDNA copies/L of air
L_u	Average upstream viral load, ssDNA copies/L of air
t	Sampling duration, min
T_{inlet}	Inlet temperature, °C
T_{outlet}	Outlet temperature, °C
ΔT	Temperature rise, °C
$\eta_{i,filter}$	Air filter efficiency, %
v	Sample volume, mL

CHAPTER 1 INTRODUCTION

Aerosol transmission of swine disease pathogens has been proven as significant risk factor on the health and productivity in the swine industry. Two of economically significant pathogens of the porcine respiratory tract, porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (*M hyo*), have been reported to be capable of long-distance airborne transport to 4.7 km and 9.1 km (Dee et al., 2009 and Otake et al., 2010). Similarly, influenza A virus (IAV) in pigs was found capable of being exhausted from swine barns and transported downwind (Corzo et al., 2013). These pathogens can infect a swine herd and their interaction can cause porcine respiratory disease complex (PRDC) which is one of the most costly diseases for intensive swine production worldwide (Bourry et al., 2015). Diseases caused by these pathogens can impact the swine industry through actual loss in animal productivity, added costs of medication and eradication measures, and even potential loss of access to markets for pigs from a PRRS-positive herd. A study in the U.S. estimated the annual financial impact of PRRS at US$664 million attributed to combined productivity losses in the breeding and grow-finish pig herds (Holtkamp et al., 2011). Similarly, a Canadian study estimated economic losses in a breeding facility affected by PRRSV to be from $250 to $460/sow/year for chronic PRRS or new acute outbreak (Mussell, 2010 as cited by Pouliot et al., 2013). As substantial investments have been made by producers on control strategies to address the multiple routes of these porcine respiratory tract diseases, it has become apparent that residual risk of infection is associated with airborne transmission (Dee et al., 2012). This led to various studies that provided evidences of the efficacy of incorporating air filtration systems making use of mechanical filters and antimicrobial filters on swine barn facilities and eradication of loopholes in the said disease prevention strategy particularly on the North American swine production system (Alonso et al., 2012; Dee et al., 2010; Dee et al, 2012; Alonso et al., 2013). However, no study has been published on evaluation of effectiveness of an air filtration system installed on a fully-operational mechanically ventilated swine transport trailer for prevention of disease infection via the airborne route during transport.

A study completed on this subject (Predicala and Alvarado, 2014) showed that the use of filtration systems using MERV 16 and Noveko bag filters can effectively capture bioaerosols in the air and prevent entry into the animal compartment of the trailer, thereby protecting the animals from potential infection by airborne transmissible diseases during transport. However, the final design was not implemented to a commercial swine transport trailer loaded with pigs to determine the impact of the air filtration system on air quality and thermal environment inside the trailer.

Pork production is a major industry in Canada (Dorjee et al., 2013) and its success lies on highly improved breeding stock. These breeding stocks are transported from pig genetics companies whose nucleus and multiplier farms are located in various provinces in the country where disease pressure is low and biosecurity perimeters are wide. While in transit, these high-value genetic stocks are inevitably exposed to risk of airborne disease contamination. Several Canadian studies have provided evidence that introduction of infected animals, particularly gilts and sows into farms, was one of the common reasons for spread of PRRSV in the country (Kwong et al., 2013; Rosendal et al., 2014; Thakur et al., 2015). Thus, it is imperative that measures be developed to prevent infection of these animals during transport and consequently close the biosecurity gap through which potential infection can be introduced to a much bigger commercial swine herd. Combined with the growing pressure on the trucking industry to provide more humane vehicles (i.e., capable of providing stable, acceptable environmental conditions and minimizing incidence of fatigued animals, among others), these created a significant challenge to transporting pigs nowadays.

The hypothesis for this study is that an efficient air filtration system in conjunction with an effective mechanical ventilation design fitted to a commercial swine transport trailer will prevent threats to animal health via airborne route and improve animal micro-environment during transport, both of which are among major concerns for existing swine transport trailers.

CHAPTER 2 LITERATURE REVIEW AND RESEARCH OBJECTIVES

2.1 Airborne transmissible swine diseases and their economic impact

In swine production, epidemiological study of pathogens capable of aerosol transmission has been done to come up with appropriate preventive actions. The important viral swine diseases that spread via aerosols are porcine reproductive and respiratory syndrome (PRRS), influenza A (IA), foot and mouth disease (FMD), enzootic pneumonia, classical swine fever (CSF), Aujeszky's disease (AD) and porcine epidemic diarrhea (PED) (Stärk, 1999). These diseases had caused overwhelming losses in the swine industry especially for those located in high density swine production areas. Moreover, options to restrict the spread of airborne pathogens are limited (Alonzo et al., 2015).

Porcine reproductive and respiratory syndrome has become one of the most significant diseases of global intensive swine production (Plagemann, 2003; Pileri and Mateu, 2016). Its causative agent, PRRS virus (PRRSV) is a systemic small, enveloped, single-stranded RNA virus of the *Arteriviridae* family and replicates in alveolar macrophages (immune cell in the lungs). The virus' diverse clinical manifestations can lead to dramatic production losses due to reproductive failure, severe pneumonia in neonatal and nursery piglets, decline in average daily gain and feed efficiency, and increased mortality for pre-weaning and growing pigs (Neumann et al., 2005; Zimmerman et al., 2012). The virus has been demonstrated capable of airborne transport as far as 9.1 km from source herd (Otake et al., 2010). Holtkamp et al. (2013) calculated combined losses of US$664 million/year in USA's national breeding and growing-pig herds due to more virulent PRRSV strain and increased disease prevalence. In Ontario, Canada alone, annual estimated cost due to PRRS was $36-$73 million (Mussell et al., 2011 as cited by Rosendal et al., 2014).

Considered important because of its zoonotic potential is influenza A virus (IAV) (*Orthomyxovirideae* family) which is shed in respiratory secretions of diseased pigs. Airborne transmission of IAV has been demonstrated for humans (Brankston et al., 2007), mice (Schulman, 1967), guinea pigs (Mubareka et al., 2009), and chicken (Yee et al., 2009). Moreover, dispersion

models incorporating particle size characteristics revealed plausibility of IAV transmission via airborne route in public places settings (i.e. healthcare, day care) (Stilianakis and Drossinos, 2010). Corzo et al. (2013) provided evidence of the likelihood of aerosol transmission of IAV in pigs under field conditions. In pigs, swine flu is characterized by fever, depression, sneezing, coughing, rhinorrhea, lethargy, and abortions to sows in febrile state (Olsen et al., 2006). The decrease in growth resulting to increased variation in pig weights is among the negative impacts of the disease which is characterized by low mortality but high morbidity.

Foot and mouth disease is a highly communicable disease of the cloven-hoofed livestock whose agent FMD virus (FMDV), is of the *Picornaviridae* family (Grubman and Baxt, 2004). Clinical signs include loss of appetite and milk production, fever, depression, lesions on foot and blister-like sores on the tongue, lips, in the mouth, teats, and between hooves. The disease has been reported capable of airborne transport up to 60 km over land and up to 280 km over the sea (Donaldson et al., 1982). Similar to several other countries in the world, Canada is considered free of FMD and reported its last FMD case in 1952 (CFIA, 2018).

Similar to swine influenza, enzootic pneumonia caused by bacterial pathogen *M hyo*, is characterized by high morbidity but low mortality (Maes et al., 1996). The primary clinical sign of the disease by itself is sporadic dry coughing that is not associated with decline in growth performance for grow-finish pigs (Straw et al., 1989). However, infection by *M hyo* occurs more commonly as part of PRDC. Enzootic pneumonia as part of the disease complex is associated with decrease in average daily weight gain and feed efficiencies in growing pigs (Bourry et al., 2015). *M hyo* is transmitted both by direct contact and by aerosol. Otake et al. (2010) provided evidence of the airborne transport of *M hyo* up to 9.2 km from a source herd. Earlier epidemiological studies on the disease recommended a 3.2 km discriminating distance from an infected herd to a farm maintaining a *M hyo*-free status (Goodwin, 1985).

Classical swine fever or hog cholera results from infection by CSF virus (CSFV) of the *Flaviviridae* family. In the acute form, common clinical signs are high fever, lethargy, anorexia, conjunctivitis, diarrhea, and purple discoloration of the snout, ears, and tail. CSF is most commonly transmitted through direct contact while aerosol transmission remained equivocal but has been demonstrated experimentally (Dewulf et al., 2000). The disease has been reported in most countries around the world especially Asia and North and Central America. Fortunately, Canada

and some other countries like United States, New Zealand, Australia, and Switzerland were able to successfully eradicate the disease (CFIA, 2018).

Aujeszky's disease (pseudorabies) is another highly contagious disease causing neurological and respiratory disease in swine. Infection is caused by AD virus (ADV) of the *Herpesviridae* family and route of transmission is wide including the airborne route. In a study by Grant et al. (1994), long-distance transport of ADV has been predicted to occur between farms 1.3 to 13.8 km away. The disease was endemic in the United States; however, an eradication campaign succeeded at eliminating the disease from domesticated pigs. Feral pigs, though, caused concerns of re-transmission to domestic herds. Canada, a number of European countries, and New Zealand has eradicated the disease from their domesticated herds (CFSPH, 2006).

Lastly, PED virus (PEDV) (*Coronaviridae* family) is an enteric virus that replicates primarily in small intestines, concentrated on feces and can also replicate in alveolar macrophages (Park and Shin, 2014). PED is an emerging and re-emerging disease worldwide (Lee, 2015) that causes watery diarrhea, vomiting, anorexia, and depression. Morbidity could reach 100% and mortality rate at 50-100% for piglets up to one week old and become less severe with older pigs, including sows. Infection is principally through the fecal-oral route. However, a study by Alonso et al. (2014) wherein they assessed whether PEDV can become airborne and remain infective provided evidence of potential risk from aerosol transmission of the disease.

2.2 Airborne transmission of swine pathogens

2.2.1 Size distribution, concentration, viability in the environment and transport of pathogen-laden particles

Fernstrom and Goldblatt (2013) cited Gregory (1973) definition of aerobiology as the study of the mechanisms in which microorganisms are moved from one geographical location to another. It includes study of aerosolized transmission of pathogens which could either be in droplet or airborne form. Droplet transmission refers to disease transmission where expelled particles settle to the surface quickly and typically within 3 feet from the site of generation. On the contrary, airborne transmission is the transmission of pathogen-laden particles that are relatively smaller in size and has the propensity to remain suspended in the air for longer periods of time. To delineate between the two, the World Health Organization employs particle diameter of 5 μm such that particles ≤ 5 μm in diameter are classified under airborne transmission while particles > 5 μm in

diameter are under droplet transmission (Cole and Cook, 1998). The succeeding sections focus on airborne disease transmission which the air-filtration system fitted to the livestock transport trailer in this study aims to address.

Airborne transmission of swine pathogens especially in pig dense regions was unclear due to multiple possible routes of infection. However, a number of field and experimental studies have proven long-distance transport and transmission of economically significant swine pathogens (Mortensen et al., 2002; Dee et al., 2009; Otake et al., 2010; Corzo et al., 2013; Alonso et al., 2015). Despite being recognized as an important route of disease transmission, the airborne route is still not well understood (Turgeon et al., 2014). Thus, studies aimed to understand the aerosolization process, preservation of pathogen infectivity while airborne, and standardization of the air sampling methods are still being conducted (Verrault et al., 2008; Gralton et al., 2011). The field of study that aimed to achieve the above-mentioned subjects on viral pathogens is called aerovirology (Verrault et al., 2008).

Viruses need host cells to multiply. They would adhere to particles of different natures such as fecal materials, respiratory fluids, water, dust, debris, bedding or hair particles found within an animal housing and can then be aerosolized in many ways ranging from wind to human or animal activities such as sneezing, coughing or mechanical processes (Alonzo et al., 2015). The bioaerosols released by a sick animal are of various particle sizes. The lower size limit would be the size of the pathogen itself (Table 2.1) while the upper limit depends on the size of the particle on which the pathogens are attached (Verrault et al., 2008). In fact, bioaerosols are of diameter sizes between 0.5 to 100 µm (Hirst, 1995 as cited by Batista et al., 2008). Verrault et al. (2008) emphasized that the means of aerosolization has a direct impact on the aerodynamic size of the aerosol.

The size distribution and aerosol composition (organic and inorganic) determine the location in which deposition of infectious agents will occur (Zuo et al., 2013; Alonzo et al., 2015). Particle size affects the time the infectious agents can remain airborne, the distance it can be transported as well as the survivability and viability of the pathogens. In a study by Alonzo et al. (2015), IAV, PRRSV and PEDV were detected at varying concentrations (in RNA copies/m^3) in all size ranges ranging from 0.4 to 10.0 µm except particles between 0.7 to 2.1 µm for PRRSV. Furthermore, virus viability was demonstrated for PRRSV and IAV in aerosol sizes greater than 2.1 µm. These

support the findings of Zuo et al. (2013) on the association of increased infectivity and concentration with increase in particle size of the aerosol. This knowledge on particle sizes is necessary in the design of preventive actions (e.g., air filtration) and experimental plan in aerovirology and in the selection of personal protective equipment for humans. Moreover, according to Cole and Cook (1998), the airborne transmission of disease pathogens is dependent on the interplay of several factors, primarily particle size and the extent of desiccation.

Table 2.1. Common swine diseases than can be potentially spread airborne.

Swine Disease	Causative Agent	Epidemiological Characteristics[a]	Reference(s)
Porcine reproductive and respiratory syndrome	Porcine reproductive and respiratory syndrome virus (PRRSV)	*Arteriviridae*, 50-65 nm, enveloped, ssRNA	Cavanagh, 1997
Influenza A	Influenza A virus (IAV)	*Orthomyxoviridae*, 80-120 nm, enveloped, ssRNA	Olsen and Brown, 2006
Classical swine fever (hog cholera)	Classical swine fever virus (CSFV)	*Flaviviridae*, 40-60 nm, enveloped, ssRNA	Thiel et al, 1996 as cited in Summerfield and Ruggli, 2015
Foot and mouth disease	Foot and mouth disease virus (FMDV)	*Picornaviridae*, 25-30 nm, non-enveloped, ssRNA	Belsham, 1993
Aujeszky's disease (Pseudorabies)	Aujeszky's disease virus (ADV)	*Herpesviridae*, 200 nm, enveloped, dsDNA	Schoenbaum et al., 1990 (as cited in Verrault, 2008)
Enzootic pneumonia	*Mycoplasma hyopneumoniae* (*M hyo*)	*Mycoplasmataceae* (bacteria), 400-1200 nm, no cell wall	Tajima and Yagihashi, 1982
Porcine epidemic diarrhea	Porcine epidemic diarrhea virus (PEDV)	*Coronaviridae*, 95 - 190 nm, enveloped, ssRNA	Pensaert and de Bouck, 1978 (as cited in Lee, 2015)

[a]ssRNA, single-stranded RNA; dsDNA, double-stranded DNA.

Another important factor that determines movement and deposition of pathogen-laden particles is rate of desiccation (Cole and Cook, 1998). Large, moisture-laden particles can desiccate quickly (Wells, 1934 as cited by Fernstrom and Goldblatt, 2013). Consequently, the particle can become smaller and lighter and stay airborne longer. Also of concern are very large

aerosol particles that initially drop on the surface only to become airborne again after desiccation (Cole and Cook, 1998).

Cole and Cook (1998) pointed out concentration of the pathogen, the infectious dose and virulence of the organism as determinants of the ability of the pathogen to cause infection. For instance, Corzo et al. (2013) were able to detect and quantify IAV from four acutely infected pig barns in Southern Minnesota and Northern Iowa. Average viral loads tested by RT-PCR were 3.20×10^5 RNA copies/m^3 for barn interior samples, 1.79×10^4 RNA copies/m^3 for exterior exhaust fan samples and 4.65×10^3 RNA copies/m^3 for downwind samples taken at 1.5 and 2.1 km away from the infected farms. Similarly, Alonso et al. (2015) characterized the concentration and particle size distribution of airborne particles carrying IAV, PRRSV and PEDV. Varying concentrations of the viral pathogens were detected: 5.5×10^2 to 4.3×10^5 RNA copies/m^3 for IAV, 6×10^2 to 5.1×10^4 RNA copies/m^3 for PRRSV and in higher quantity at 1.3×10^6 to 3.5×10^8 RNA copies/m^3 for PEDV. These viral loads were determined at particle sizes ranging 0.4 μm to 10 μm. On the other hand, the minimum infectious dose (MID) of PRRSV according to Pileri and Mateu (2016) depends on the route of exposure, the dose, and the type of PRRSV isolate involved. Hermann et al. (2005 and 2009) found that the infectious dose 50 (ID_{50}) via oral, intranasal, intramuscular and aerosol exposure routes are $10^{5.3}$, $10^{4.0}$, $10^{2.2}$ and $10^{3.1}$ $TCID_{50}$, respectively, for PRRSV 2 isolate VR-2332. Meanwhile, Yoon et al. (1999) reported that it only requires ≤ 10 particles of PRRSV genotype 2 isolate ISU-P to infect pigs parenterally.

A combination of factors such as relative humidity (RH), temperature, ultraviolet (UV) radiation, aerosolization medium, exposure period, composition of the surrounding air and composition (organic and inorganic) of the pathogen-laden particle affect its infectious potential (Verrault et al., 2008). RH has been highly investigated as a factor that affects infectivity of airborne viruses. Preservation of infectivity depends on the type of virus. Some may require low RH (below 30%), an intermediate RH (30% to 70%) or a high RH (over 70%). For instance, IAV (Harper, 1961), Newcastle disease virus (Songer, 1967), and PRRSV (Hermann et al., 2007) which are enveloped pathogens are most stable at low RH. On the contrary, most of the non-enveloped viruses such as rhinovirus, poliovirus and picornavirus (Akers and Hatch, 1968) are most stable at high RH. Enveloped human coronavirus 229E and pseudorabies virus (Schoembaum et al., 1990) and non-enveloped when mature rotavirus (Ijaz et al., 1987) are most stable at intermediate RH. The study of Akers and Hatch (1968) on airborne picornavirus, however, indicates that there is no

outright correlation between RH and the preservation of the infectivity of an airborne virus. Nevertheless, generally low RH preserves stability of enveloped viruses, while high RH best preserves the infectivity of non-enveloped viruses. Furthermore, Gralton et al. (2011) reported that the effect of RH on the viability of a pathogen on a carrier particle is that low RH causes desiccation and can make the particle smaller from evaporation.

Temperature and UV radiation can also impact airborne virus infectivity. The infectivity of pseudorabies (Schoembaum et al., 1990), PRRSV (Hermann et al., 2007), and IAV (Harper, 1961) are increased at low temperatures. In a study by First et al. (2007), UV germicidal lamps were used to inactivate airborne organisms such as *Serratia marcescens*, *Bacillus subtilis* spores and vaccinia virus. The method of aerosolization has also shown potential to inactivate some viruses depending on its medium, the temperature, and RH (Ijaz et al., 1976). The aerosolization and sampling set-up used by Verrault et al. (2010) reported impact on the infectivity of the recovered aerosolized phages (Phi X174 and P008). Similarly, result of the test conducted by Turgeon et al. (2014) comparing three nebulizers (TSI 9302 atomizer, Aeroneb Lab nebulizer and Collison 6-jet nebulizer) in the aerosolization of the dsDNA phage PR772 suggested that the aerosolization as well as the sampling method used affects the structural integrity of the surrogate virus. Moreover, the chemical composition of the particle on which the pathogen is attached has different effect on the stability of the aerosolized pathogens (Verrault et al, 2008). For instance, salt added to the spray suspension reduced the recovery of infectious Semliki Forest virus in a controlled chamber experiment. In contrast, use of an organic fluid (allantoic fluid) in the nebulization buffer increased the relative recovery of phage Phi 6 while it did not significantly affect the recovery of infectious phages MS2, PM2 and Phi X174 (Turgeon et al., 2014). Organic fluids and other chemical compounds according to Verrault et al. (2008) perform their protective effect by limiting desiccation and other environmental stresses. Finally, composition of the ambient air, such as ozone concentration has been shown to be highly effective in inactivation of airborne bacteriophages MS2, Phi X174, Phi 6 and T7 (Tseng and Li, 2005).

Apart from the direct effect of climatic conditions on the infectivity and stability of pathogens, other factors such as temperature, RH and wind velocity impact the aerosol transport of the pathogens from one location or host to another. For instance, the spread of influenza around the world has been given much attention in recent years to understand its pathogen's stability, transmissibility and seasonality. Pigs are major carriers and are considered mixing vessels of the

zoonotic influenza virus (Webster et al., 1992). The 2009 H1N1 pandemic was a triple assortment of avian, swine and human influenza virus (Garten et al., 2009). Several studies have investigated the seasonal dependence of the pathogen. Temperate regions of the northern and southern hemispheres have shown regular seasonal pattern, i.e. during their respective winter months (Viboud et al., 2004). Nelson and Holmes (2007) in their review of the evolution of the epidemic influenza presented the weekly reports on influenza-like illness from the World Health Organization (WHO) FluNet surveillance system. The report depicted influenza virus activity peaks during winter until early spring in countries at similar latitude in the northern hemisphere; during late spring and early summer in the southern hemisphere; and, for countries closer to the equator, influenza often occurs year-round. Similarly, Tamarius et al. (2013) explored the association between influenza seasonality and climate in 78 representative global sites. By modelling epidemiological and climatic information from the sample locations, it was validated that during the "cold-dry" months (i.e. winter) when temperature and specific humidity are low that virus activity peak. Additionally, temporal and spatial dynamics of PRRSV as depicted in a four-year study in the United States showed weekly incidence of the disease as low during spring and summer and high during fall and winter (Tousignant et al., 2014).

Several studies have shown the role of humidity and temperature on the transmission of influenza virus. Using a guinea pig model, Lowen et al. (2008) investigated the likelihood of influenza transmission in environmental conditions typical of tropical regions. It was observed that at 30°C and various RH levels (20%, 50%, 65%, and 80%), no aerosol transmission of the virus occurred as evidenced by no virus detected on nasal washings from exposed guinea pigs. Moreover, virus titers were examined from nasal washings of inoculated pigs at both 30°C and 20°C and no significant difference on the amount of virus shed was observed. This indicated that the absence of transmission at higher ambient temperature was not due to the decrease in the amount of virus shed by the inoculated guinea pigs. On the other hand, transmission occurred at 30°C when infected and naïve guinea pigs are placed in the same cage. The study revealed that the sensitivity of the influenza virus to RH and temperature influence its mode of transmission. It suggests that aerosol transmission can govern transmission in temperate regions while transmission is predominantly through direct contact in tropical regions.

2.2.2 Surrogate organisms in bioaerosol studies

Although important information can be derived from using the pathogen itself in aerobiological studies, the risk of exposure is high for individuals involved. Therefore, surrogate viruses such as those given in Table 2.2 are used to mimic the behavior of the pathogens. Bacterial viruses, or bacteriophages, pose no significant risk to humans, are relatively easy to produce without requiring strict biocontainment precautions, and the large pool of surrogate viruses to choose from enabled some phages to display properties similar to eukaryotic viruses (Tseng and Li, 2006; Gendron et al, 2010; Turgeon et al., 2014).

Table 2.2. Common viral aerosol models.

Viral Strain[a]	Growth Conditions and Characteristics[b]	Reference(s)
ATCC 15597-B1 or HER-462	MS2, *Leviviridae*, 25 nm, non-enveloped, icosahedral, tail-less, ssRNA, bacterial host *Escherichia coli* (HER 1462)	Golmohammadi et al., 1993
ATCC 21781-B1 or HER 102	Phi 6, *Cystoviridae*, 85 nm, enveloped, spherical, tail-less, dsRNA, bacterial host *Pseudomonas syringae* var. *phaseolicola* (HER 1102)	Ellis and Schlegel, 1974
ATCC 11303-B7 or HER 30	T7, *Podoviridae*, 45 nm, non-enveloped, icosahedral, tailed, dsDNA, bacterial host *Escherichia coli* (HER 1024)	as cited in Verrault et al., 2008
ATCC BAA-769-B1 or HER 221	Phage PR772, *Tectiviridae*, 80 nm, non-enveloped, icosahedral, tail-less, dsDNA, bacterial host *Escherichia coli* (HER 1221)	Lute et al., 2004
HER 228	Phage P008, *Siphoviridae*, 53 nm, isometric, tailed (159 nm non-contractile tail), dsDNA, bacterial host *Lactococcus lactis* F7/2A (HER 1228)	Jarvis et al., 1991
ATCC 13706-B1 or HER-036	Phi X174, *Microviridae*, 25-27 nm, non-enveloped, icosahedral, tail-less, ssDNA, bacterial host *Escherichia coli* (HER 1036)	Sanger et al. 1978 (as cited in Verrault et al., 2010)

[a]ATCC, American Type Culture Collection (www.atcc.org); HRE, Félix d'Hérelle Reference Centre for Bacterial Viruses (www.phage.ulaval.ca).
[b]ssRNA, single-stranded RNA; dsRNA, double-stranded RNA; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA.

Phage MS2 is among the most frequently used surrogate viruses in bioaerosol research studies (Gendron et al., 2010). It is a small non-enveloped virus of a genome made of single-stranded RNA (ssRNA) and a capsid diameter of approximately 25 nm. It has no tail and is

morphologically similar to members of the *Picornaviridae* family which includes pathogenic viruses such as poliovirus, rhinovirus and FMDV (Verrault et al., 2008). Phi 6 is an enveloped, tail-less, and 85 nm virus of the double-stranded RNA (dsRNA) genome. Its fragile lipid containing envelope resembles that of most of the viruses listed in Table 2.1. PRRSV being an enveloped virus, for instance, is sensitive to temperature and pH while exposure to detergents and lipid solvents such as chloroform and ether are efficient in disrupting the envelope and inactivating replication (Benfield et al., 1992). Similarly, the envelope of Phi 6 has direct impact on its sensitivity to aerosolization, air sampling process and also environmental conditions to which it is exposed (Tseng and Li, 2005). Consequently, it is used infrequently in aerosol studies especially those that require evaluation of viral infectivity (Gendron et al., 2010). Moreover, its host *Pseudomonas syringae* var. *phaseolicola*, is a known plant pathogen specifically causing halo blight in beans (Ferńandez-Sanz, et al., 2016). Thus, Phi 6 was not chosen as model virus in this current study. Tailed phage, T7, is among the earliest surrogates used. It is non-enveloped, with genomic material of double-stranded DNA (dsDNA) and its tail for host recognition makes it susceptible to physical damages. Moreover, the morphological characteristic of this phage does not resemble that of any mammalian viruses (Verrault et al., 2008). PR772 is an icosahedral dsDNA bacteriophage of the *Tectiviridae* family and shares similar properties as mammalian adenoviruses (Benson et al., 1999). PR772 has been recognized by the U.S. Food and Drug Administration (FDA) as a viral model for testing larger-pore-size virus-retentive filters in the biopharmaceutical industry (Lute et al., 2004). P008, on the other hand, is a virulent phage of *Lactococcus lactis* with a dsDNA genome, an isometric capsid of diameter approximately 53 nm and non-contractile tail 159 nm in length (Jarvis et al., 1991). In an investigation conducted by Müller-Merbach et al. (2005) on the thermal inactivation of lactococcal phage responsible for the fermentation failure of milk, Phage P008 appeared to be among the most heat resistant.

In this study, phage Phi X174 was used as representative virus of common swine pathogens. With morphology similar to MS2, phage Phi X174 is of the *Microviridae* family, non-enveloped, tail-less, 25-27 nm in size and has a single-stranded DNA (ssDNA) genome. It has been used repeatedly as a surrogate for human and animal viruses in many studies (Tseng and Li, 2005; Tseng and Li, 2006; Verrault et al., 2010; Turgeon et al., 2014) and has also been compared to highly resistant human viral pathogens, polioviruses and parvoviruses (Rheinbaben et al., 2000). Lastly,

Verrault et al. (2008) mentioned that since all viruses respond uniquely to environmental factors, therefore, no surrogate is perfect.

2.3 Application of air filtration in the swine industry

2.3.1 Biosecurity in the swine industry

Seedorf and Schmidt (2017) defined biosecurity in livestock industry as the set of measures implemented to reduce introduction and spread of infectious agents. It involves interventions to limit if not eliminate risk of contamination through different entry routes into the farm which includes pig introduction, semen, transport vehicles, humans, inanimate objects (i.e., shoes, clothes, supplies and equipment) and other animals and insects.

With the acute outbreak of PRRS in North America that caused significant financial impact (Neumann et al., 2005; Holtkamp et al., 2011), management and biosecurity procedures including gilt pool management and acclimation (Dee et al., 1995), vaccination (Cano et al., 2007), transport and insect control (Otake et al., 2002; Dee et al., 2004) and herd closure (Torremorell et al., 2003) were developed (as cited in Alonso et al., 2013). However, as multiple routes of disease transmission are being addressed, it has become evident that there is a loophole in the biosecurity efforts (Alonso et al., 2013). Thus, following several experimental, field, and epidemiological investigations that have demonstrated the potential risk of swine disease pathogen transmission through the airborne route, particularly for PRRSV and *M hyo*, producers leaned towards the potential of air filtration as a means to prevent infection and re-infection of swine herds (Dee et al., 2011; Alonso et al., 2013).

2.3.2 Air filtration systems adopted in swine barns

The main goal of installing air filtration systems in swine farms was to prevent transmission of PRRSV between farms (Alonso et al, 2013) and earlier studies conducted has proven its efficacy in preventing PRRSV infections (Dee et al., 2012). Ricard and Pouliot (2013) reported that in 2013 there were approximately 30 barns in Canada and 98 in the United States in 2012 equipped with air filtration systems. In fact, the first commercial herds in North America equipped with filters were in Quebec in 2003.

For an air filtration system to be effective in preventing entry of airborne pathogens, it is important to have an understanding of how the virus or bacteria presents itself. A virus-laden

particle according to Verrault et al. (2008) can be a complex mixture of salts, proteins, organic and inorganic matter and the virus particle itself, thus, it is necessary to realize that the size of the virus itself does not rule the airborne particle size. In a study by Hogan et al. (2005), it was shown that the particle size distribution of an artificially produced submicrometer and ultrafine aerosol of a culture media was not affected by the presence of bacteriophages. As mentioned previously, Batista et al. (2008) cited Hirst (1995) stating bioaerosols are of diameters varying from 0.5 to 100 μm, making the interception of these virus or pathogen-laden particles by HEPA or other types of filters possible.

Two types of filters were used for the swine sector: mechanical filters which capture airborne particles when they encounter the filter media and adhere to its fibres; and, antimicrobial filters that are made of polypropylene fibers embedded with antimicrobial agents that inactivate viruses upon contact (Ricard and Pouliot, 2013). The American Society of Heating, Refrigerating and Air-Conditioning Engineers Inc. (ASHRAE) published a standard for filter efficiency. The gravimetric ASHRAE method measures efficiency of pre-filters used to retain larger dust particles. Efficiency values from this method are based on the mass of dust not captured by the filter. Therefore, higher values mean less dust particles captured. ASHRAE's opacimetric method on the other hand, rates air filter effectiveness in Minimum Efficiency Reporting Value (MERV) given on a scale of 1 to 16. A higher rating value signifies greater effectiveness of a filter in trapping fine particulate matter. This rating method is used on high efficiency filters including those commonly used in the hog industry. Also, the ASHRAE dioctyl phthalate (DOP) test is used to rate extremely high efficiency filters such as high efficiency particulate arrestance or HEPA filters. The ASHRAE standards, however, are applied only to mechanical filters while antimicrobial or antiviral filters have no standard rating (Pouliot et al., 2011).

In an investigation by Dee et al. (2005), a three-stage air filtration system was tested for potential in preventing PRRSV infection. The system consisted of a 20% gravimetric wire mesh pre-filter, an EU 8 rated bag filter with 95% opacimetric rating and a 99.99% DOP rated HEPA filter classified as EU 13. The results suggested that the air-filtration system evaluated was highly effective in preventing aerosol transmission of the disease. None out of 20 pigs became PRRS-positive in the filtered set-up whereas 6 of 20 were infected in the nonfiltered facility. This system, however, required a positive-pressure ventilation system which was costly, preventing many producers from considering installation of HEPA filtration.

Research studies continued to find more cost-effective air-filtration systems. Thus, Dee et al. (2006a) conducted an evaluation of three filtration methods having potential to reduce PRRSV transmission through aerosol: HEPA filtration, an irradiation system through ultraviolet light (UV) and a low-cost filtration consisting of mosquito netting (pre-filter), a fiberglass furnace filter (MERV 4), and an electrostatic furnace filter (MERV 12 rated EU 3). It was found that HEPA filter was significantly more efficient than the other two methods because no pig became positive for PRRSV. The low-cost filtration system just like HEPA filtration significantly reduced PRRSV transmission having only 4 out 10 pigs infected compared to 8 out of 10 for the UV irradiation and 9 out of 10 for the control (no air filtration).

A four-year study on the efficacy of air filtration system for the prevention of PRRSV and *M hyo* was done by Dee at al. (2011) using a four-building neighborhood model. One building served as source of PRRSV and *M hyo*-positive bioaerosols while two are treatment buildings equipped with various air filtration system designed to reduce risk of aerosol transmission and the other one served as control (no air filtration system). This study reported that airborne transmission of PRRSV and *M hyo* occurred 43% and 34%, respectively, for the control building while no evidence of infection was documented in the treatment rooms. Furthermore, a long-term sustainability study by Dee at al. (2012) demonstrated the positive effect of air filtration in reducing risk of PRRSV infection on a large number of herds (38) over a longer period of time. Introduction of new PRRSV infections were significantly lower for filtered herds than the non-filtered breeding herds. Finally, the potential for PRRSV infection was 7.97 times higher before filtration was installed than after filtration was initiated.

Use of air filters with antimicrobial properties were investigated in studies conducted in Canada. Batista et al. (2008) developed an air filtration system with three filtration stages: a pre-filter for coarse particles made of detachable mosquito net, a three-layer antimicrobial filter and a second set with seven layers of the same antimicrobial filters. Evaluation of the performance of the air filtration showed 95% success rate in preventing introduction of PRRSV in a controlled environment chamber study. Additionally, Pouliot et al. (2013) reported tests results of an innovative biocontainment system for a swine quarantine facility that features use of 15 layers of antimicrobial filters installed in the attic air inlets and a three-stage filtering system at the exhaust vents: two types of pre-filters (StuffNix or MERV 13), another type of pre-filter (Noveko), and a 10-layer antimicrobial filter. These in conjunction with an ionization dust abatement system

showed better performance of the MERV 13 and antimicrobial filter combination (58 to 98% capture of dust particles depending on particle size and 96 to 98% of bacteria) over use of StuffNix as pre-filter. Under actual swine barn condition, the study emphasized importance of pre-filtering coarse particles to maximize potential of antimicrobial filters. Furthermore, evaluation of MERV 16 and a Noveko antimicrobial filter installed in a goose-neck livestock trailer showed average percent reduction in bacteriophage concentration of 89.3% and 99.8%, respectively (Predicala and Alvarado, 2014).

2.3.3 Financial implications of air filtration in swine barns

As mentioned previously, costs associated with installation of air filtration systems to swine barns limited its adaptation. Parent (2004) (as cited by Batista et al., 2008) reported that the Swine Insemination Centre of Quebec (a total of 550 boars) spent more than $1 million on purchases and retrofitting required to install a HEPA filtration system. Moreover, estimated cost for installation of a similar air filtration system (Desrosiers, 2005 as cited by Batista et al., 2008) was $537 – $1,075 per boar in an artificial insemination center and $1,075 – $1,612 per sow in a farrow-to-finish farm.

A "HEPA-like" filtration system, 95% DOP for ≥ 0.3 μm, designed and installed in some farms in the US was found effective in preventing introduction of PRRSV, IAV and *M hyo* (Dee, 2007 as cited by Batista et al., 2008). Additionally, this system has the advantage of working in a negative pressure ventilation system and costs 10% of the HEPA type filtration system. Reicks (2006) reported that the 95% DOP filtration system in an insemination center costs $32 – $107 per boar space without air conditioning or $355 – $462 per boar space with air conditioning. The HEPA filter system, on the other hand, costs $322 – $645 and $1,075 – $1,290 per boar space for without and with air conditioning, respectively. This "HEPA-like" system was composed of a 95% DOP accordion-like filter rated EU 9 and MERV 15 (Dee et al., 2006b).

More recently, Ricard and Pouliot (2013) reported estimated cost of air filtration installation in Canada. For a farrowing facility with air filter changes every five years, cost is $185 – $345 per place over a 10-year period. This cost rises to $250 – $450 per place over 10 years if filter changes are done more often than every 3 years. Cost analysis by Batista et al. (2008) for the implementation of an air filtration system in a swine barn would cost $2.02 and $2.05 per pig

produced for a 1,500 and 400 sow barn, respectively. Cost in an air-filtered nursery is $1.17 per piglet produced while the cost is $6.10 per pig produced in a finisher facility.

Alonso et al. (2013) conducted an investment analysis on three production scenarios in a hypothetical 3,000 sow farm: (1) control, (2) conventional attic filtration and (3) filtered tunnel ventilation. The payback periods based solely on sow herd productivity for scenarios 2 and 3 were 5.35 and 7.13 years, respectively. A premium of US$5 per PRRS-negative weaned pigs was found to reduce the payback periods for scenario 2 to 2.1 years and 2.8 years for scenario 3. Additionally, the filtered farm can produce 5,927 more pigs than farms with no filtration system.

Generally, costs of implementing an air filtration system depend on the level of filtration desired, the frequency of filter changes, and the changes made to the building, among other things. Profitability and return on investment depends on the frequency and intensity of the disease crisis and the herd productivity (Ricard and Pouliot, 2013).

Overall, air filtration systems applied to swine barns has significantly reduced the risk of airborne disease transmission. Although associated additional cost (capital, maintenance, operational) is high, the technology if designed effectively is still considered a cost-worthy investment in the swine industry.

2.4 Existing swine transport scenario

2.4.1 Animal welfare concerns during transport

Transportation is a large, inevitable and critical aspect of modern swine production. However, the process can be one of the most stressful stage an animal experiences that can cause behavioral and physiological changes (Brown et al., 2011). In the U.S. alone, more than 113 million pigs are transported annually (National Agriculture Statistics Service as cited by McGlone et al., 2014a). While in Canada, where hog slaughter is concentrated in the provinces of Quebec, Manitoba, Ontario and Alberta, in 2017 alone, around 21.6 million pigs were slaughtered in federally and provincially inspected facilities (Agriculture and Agri-Food Canada, 2017). The latter implies the need to transport animals produced in other provinces over a wide range of distances.

In North America, the trailers used in commercial transport exposes pigs to a multi-factor stress situation. Livestock transport in the region gained increased attention from the public, animal

rights groups, and the government for the past decade. This was escalated by the recognition of the importance of keeping good animal welfare during transport, and animal transport as a critical control point for meat quality by the World Organization for Animal Health (OIE) (Broom, 2005).

Animal welfare concerns during transport include possibility of animals experiencing stress, injury, fatigue, mortality, and morbidity which can be due to feed and water deprivation, as well as undesirable and potential extreme weather conditions inside the trailer due to trailer design, exposure to noise, vibrations and toxins, journey duration, loading densities, mixing of conspecifics, and poor animal handling (Speer et al., 2001; Ritter et al., 2006; Averos et al., 2007; Nielsen et al., 2011; Pilcher et al., 2011; Torrey et al., 2014; Xiong et al., 2015). Transport losses in market-weight pigs refer to losses due to animals stressed to various degrees and lead to non-ambulatory animals (i.e., fatigued or injured), dead on arrival (DOA), dead in yard (DIY) and dead in pen (DIP) (McGlone at al., 2012). In Canada, reported DOA reached as many as 17,000 (0.08%) pigs yearly (CFIA, 2006-2010 as cited by Schwartzkopf-Genswein et al., 2012). In addition, negative effect on meat quality (i.e., pH, change in water holding capacity, color defect) of transportation-related stress has been reported. Transport losses remained a pressing concern in the North American swine industry both in the economic and animal welfare stand point. Previous studies have documented that although several factors during transport induce stress, it is the thermal micro-environment within the transport vehicle that poses the greatest risk to the animals' welfare and well-being (Hall and Bradshaw, 1998; Mitchell and Kettlewell, 1998; McGlone et al., 2014b).

2.4.2 Existing swine trailers: Design and problems encountered

Current trailer designs used in the commercial transport of pigs in North America vary from a small single-deck trailer to large three-deck drop center trailers (also known as pot-belly). Pot-bellies are most common in North America because they can carry both cattle and a larger load of pigs (more than 200) in one journey. Downside is the multiple (up to 5) and steep (up to 40° slope) internal ramps that reduces handling ease thus encouraged use of electric pods and extended loading and unloading times (Torrey et al., 2008). Figure 2.1 shows pot-belly and straight-deck trailers. These trailers are of the dimensions close to 15.80 m long × 2.50 m wide × 3.50 m high. Small and large openings on the sides of the trailers are punched to allow passive ventilation. These

openings are covered with boards during cold weather periods at various percentages of opening. The interior trailer space is divided into different compartments or zones.

Figure 2.1. Pot belly (top) and straight deck (bottom) livestock trailers commonly found in Canada. Figures obtained from *www.robergeinc.com, www.thepigsite.com.*

The growing concern towards animal welfare during transport prompted studies to characterize the thermal environment inside transport trailers currently used in the North American swine industry under a wide range of outdoor climatic conditions. In a swine trailer micro-climate investigation done by Brown et al. (2011) in western and eastern Canada, it was found that significant temperature variations (in terms of delta temperature and delta humidity ratio) occur within both pot belly and dual, straight-deck trailers. In a pot belly, a temperature warmer by 10°C than the ambient (outdoor) temperatures was recorded on the lower front compartments. This rise in compartment temperature is extremely undesirable during hot periods considering the lower and upper critical temperature of 26 to 31°C for market pigs (20-100 kg) during transport (Randall, 1993). Results from the monitoring of trailer environment by Ellis et al. (2008) suggested similar results. In all four seasons, the temperature in the trailer increased from rear to front. In contrast to observation by Brown et al. (2011), during spring, summer and fall, temperatures in the upper deck were slightly higher than at the lower deck. Moreover, temperature extremes and change were observed during periods when the trailers were stationary: during loading, road travel interruptions, and at the plant before and during unloading (Ellis et al., 2008). These findings are

supported by studies conducted by Kettlewell et al. (2001) where peaks in pig heat production occur at loading attributed to increased animal activity. Periods of equilibration were then recorded after the trailer starts moving and continue for a period of time. Similarly, during extended stationary periods, such as enforced stops on the road, the pigs get agitated, thus, disturbing the thermal equilibration inside the trailer. Mean heat production were 1.4 W/kg and 1.9 W/kg for pigs on continuous and interrupted journeys, respectively.

Thermoneutral zone is the range of ambient temperatures within which the animals are able maintain core body temperature with the least amount of energy for thermoregulatory efforts. It is defined by the upper and lower critical limits and at which condition, animals are expected to be performing optimally and in best health status. Zulovich (2012) and Agriculture and Agri-Food Canada (1993) as cited by Brown et al. (2013) identified 10 - 21°C as thermoneutral zone for finishing pigs weighing approximately 100 kg. On the other hand, Curtis (1985) as cited by Xiong (2013) indicated 10 - 25°C as the thermoneutral range for finishing pigs. Due to developments in genetics, pig nutrition, and housing, the thermoneutral zone for finishers was modified to 18 - 28°C as reported by Brown-Brandl et al. (2013).

Having identified the causes of transport losses, particularly those associated with the design of the transport trailer, the swine industry is keen on finding solutions to provide the animals an acceptable environment during transport. Remedy encompasses putting fans on critical locations inside the trailer (Kettlewell et al., 2001; Brown et al., 2011) or varying the location and increasing side opening areas (Ellis et al., 2008) to increase ventilation rate, providing water sprinkling to encourage evaporative cooling (Brown et al., 2011), effective boarding (McGlone et al., 2014a) and bedding levels (McGlone et al., 2014b) during mild and cold weather periods. Indeed, studies have proven the effectiveness in reducing transport mortality of forced ventilation and intermittent misting systems in an animal trailer (Christensen and Barton-Gade, 1999; Chevillon, 2000). Also, Fox (2013) provided evidence of the positive effect of water sprinkling on the gastrointestinal tract temperature and trailer micro-climate during periods of high ambient temperatures.

2.4.3 Mechanical ventilation in livestock trailers

2.4.3.1 *Ventilation system effectiveness: Assessment method*

Two of the common criteria in the evaluation of the performance of ventilation systems in animal housing are ventilation efficiency and ventilation effectiveness. Zhang et al. (2001) defined

ventilation efficiency as the mass of air delivered (kg of air) per unit power consumed (watt) by ventilation system at a given pressure. It is also referred to as ventilation effectiveness factor (VEF). Ventilation effectiveness on the other hand is a direct measure for contaminant (particulate, gas, humidity or heat) removal in the air space of concern. In this study, ventilation effectiveness was used as criterion for the performance of the ventilation system of the prototype air-filtered trailer. Ventilation effectiveness can be assessed on the basis of heat and contaminant (e.g., CO_2) removed from the air space of concern, i.e., the animal occupied zone (AOZ) in animal housing. Heat removal effectiveness (HRE) and contaminant removal effectiveness (CRE) are indicators for uniform mixing and elimination of dead zones and unwanted drafts (van Wagenberg and Smolders, 2002). Values equal to 1 mean a perfectly mixed air space. However, due to air flow patterns and different heat and contaminant sources within an air space, HRE and CRE can be above or below 1 (Price et al., 1999). Values lower than 1 indicate that the temperature level in the AOZ exceeds the temperature in the exhaust and translate to high contaminant and heat levels at the AOZ that are not efficiently removed by the ventilation system. On the other hand, values above 1 translate to effective air displacement in the AOZ. At low ventilation rates common during heating periods, effective removal of contaminants (CRE>1) is desirable for acceptable indoor air quality and energy savings from less ventilation. On the other hand, an HRE>1 is desired during warm periods where ventilation rates are high and mainly controlled by temperature at the AOZ (van Wagenberg and Smolders, 2002).

2.4.3.2 *Recent developments on mechanically ventilated livestock vehicles*

Majority of past studies on thermal environment during livestock transport conducted both in Europe and North America were done on naturally (passive) ventilated vehicles (Ellis et al., 2008; Knezacek et al., 2010; Brown et al., 2011; McGlone et al., 2014b; Goldhawk et al., 2015; Xiong et al., 2015). Limited investigations explored the potential of adding mechanical ventilation systems to livestock vehicles to improve overall thermal condition during transport (Kettlewell et. al., 2001; Norton et al., 2013). According to Kettlewell et al. (2001), previous studies on fan ventilated animal transporters examined only the effect of the resultant micro-climate to the animals instead of defining the requirements for the confined system. In their study, Kettlewell et al. (2001) investigated the performance in terms of the internal thermal micro-environment of a swine prototype trailer with mechanical ventilation system in one of its decks. Heat and moisture production inside the trailer loaded with pigs were measured. At the same time, a provisional

recommended airflow rate of 0.25 m^3/s per ton live weight was suggested. However, this value was derived solely from consideration of market weight pigs transported under typical range of warm weather condition in the United Kingdom (ambient temperature of 15-28°C).

In order to optimize welfare of animals during transport, legislation and animal transport regulations particularly in Europe were put in place. In 2007, Council Regulation (EC) 1/2005 was introduced to member states of the European Union to further animal transport. The emphasis of the regulation was control of temperature and the forced ventilation (Mitchell and Kettlewell, 2008). Among its specific mandates were to ensure temperature of 5 to 30°C within the transport vehicle at a tolerance of ±5°C depending on ambient temperature. Additionally, the ventilation system must be capable of operating at a minimum airflow of 60 m^3/hr/KiloNewton payload. Furthermore, Mitchell and Kettlewell (2008) recommended purely physical determination of ventilation flow rate for animals in transport on the basis of the allowable temperature rise between ambient and interior, heat generated by the animals and thermal properties of the air. Equation 2.1 gives an estimate of the required ventilation flow rate for animal transport:

$$VFR = \frac{TMHP}{C_p \times \Delta T} \tag{2.1}$$

where VFR is the ventilation flow rate in m^3/s, TMHP is total metabolic heat production in J/s, C_p is volumetric heat capacity of air (1,226 $J/m^3/°C$), and ΔT is acceptable temperature rise in °C. This method was applied in the computation of maximum ventilation flow rate for the air-filtered trailer in this current study. In North America, on the other hand, guidelines in the transport of animals are introduced through Transport Quality Assurance (TQA) and Codes of Practice for the care and handling of farm animals. In Canada, the Canadian Food Inspection Agency (CFIA) is responsible for the humane transport of farm animals. Furthermore, Health of Animals Regulations (C.R.C., c. 296) in Canada is in place but unlike Council Regulation (EC) 1/2005, it does not have provisions on augmenting passive ventilation with fans particularly for animals transported by land (Canadian Food Inspection Agency, 2018).

It is apparent that limited information is available on the thermal environment inside an enclosed and mechanically ventilated animal trailer, more so, those operated under North American weather conditions. If air filtration system is to be added, ensuring elimination of

unfiltered air entry is the top consideration, in addition to providing sufficient, well-distributed air flow.

2.5 Development of design for prototype air-filtered trailer

This present study is part of a funded research project at the Prairie Swine Centre Inc., Saskatoon, SK, that aimed to develop an improved air-filtered trailer that can reduce risk of airborne pathogen contamination during transport and improve operational efficiencies (Predicala et al., 2017). The following sections briefly describe the design process that led to the prototype trailer assembled and evaluated as described in subsequent chapters.

2.5.1 Conceptualization of initial design

A survey that gathered inputs from relevant stakeholders (e.g., veterinarians, truckers, herdsmen, livestock producers) pointed out that (1) difficulty in loading and unloading, (2) variable thermal conditions inside the trailer, and (3) risk of airborne disease infection during transport, are among the most pressing issues with existing commercial livestock transport vehicles. Additionally, computer simulation on the conventional trailers verified the previous claims that air comes in and out of the trailer through the side openings randomly in any direction thereby exposing pigs to potential airborne disease infection and that air temperature differences between the ambient and trailer interior could reach up to 5°C. Consequently, initial trailer design featured the incorporation of an air-filtration system for airborne disease control and the provision of mechanical ventilation to better control the thermal environment inside the livestock container throughout transport.

2.5.2 Computer simulation and selection of final design

Computer simulations in the design phase of the project were done using a commercially-available computational fluid dynamics (CFD) software ANSYS (ANSYS Student License, ANSYS Inc., Canonsburg, PA). CFD has become instrumental in design and development projects in different engineering fields including in agriculture in order to reduce the number of actual physical investigations (Norton et al., 2006). In this project, six design configurations were simulated under summer conditions and the top three performing designs were further evaluated under Saskatchewan winter conditions (Predicala et al., 2017). The top-performing design in terms

of HRE and ranges of temperature, moisture, and air speed maintained at various monitoring locations inside the animal compartment was selected as final design.

Figure 2.2 shows the geometry model established using the Design Modeler module in ANSYS. The model was constructed for a completely enclosed aluminum, straight, dual-deck trailer with interior dimensions of 5.9 m × 2.35 m × 2.43 m (l × w × h). The figure also shows the options for the locations of the air inlets and outlets for the air-filtered trailer.

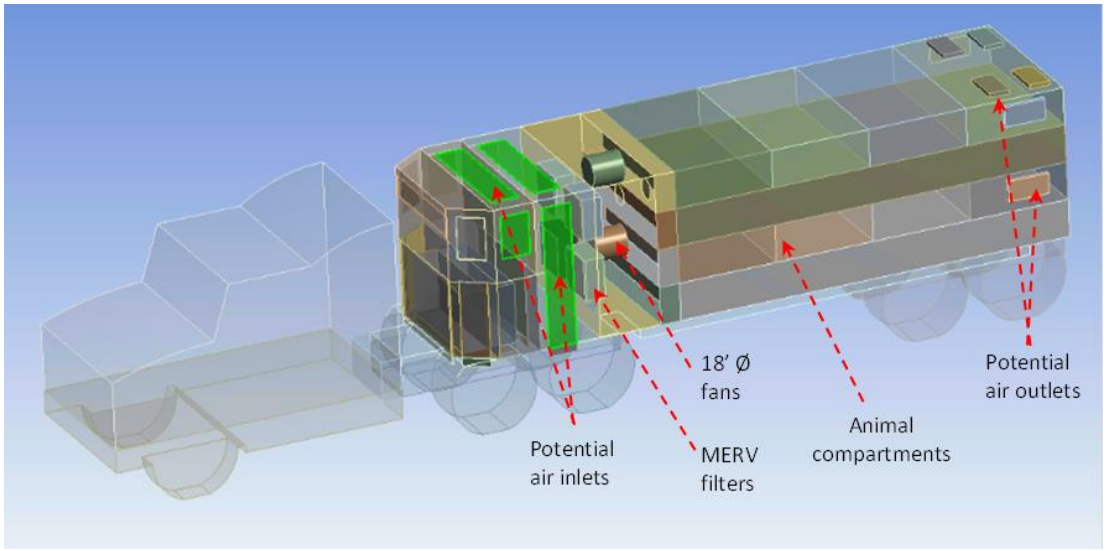
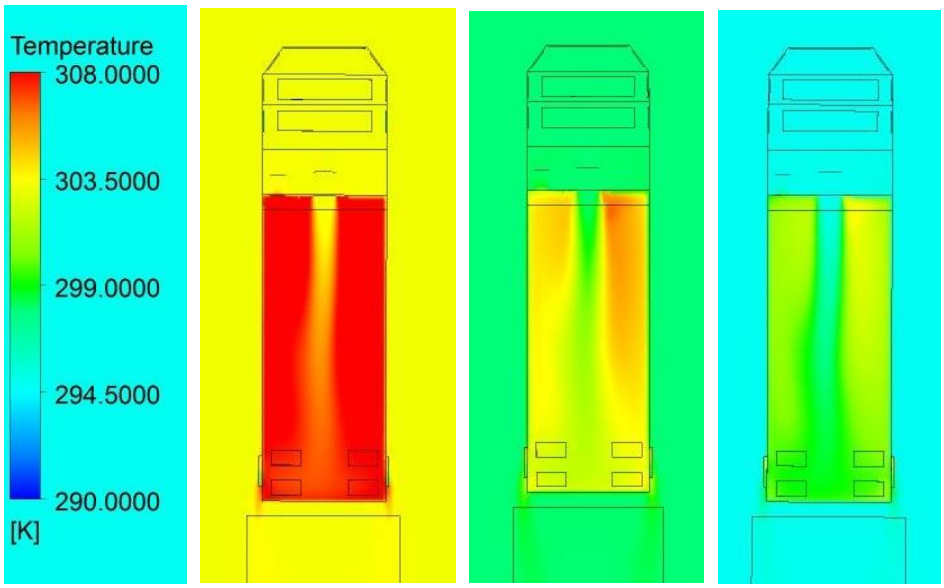


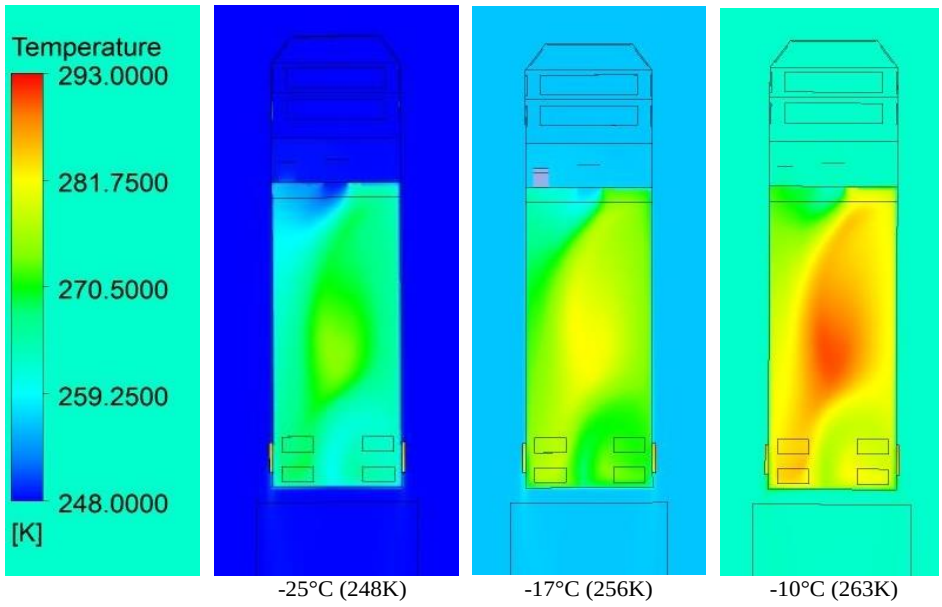
Figure 2.2. Screenshot of the geometry model of the air-filtered trailer. Figure obtained from *Predicala et al., 2017.*

Out of the six design configurations investigated, S2-S4 was selected as final design. The design is comprised of one side inlet on each side at the front of the trailer and two air outlets on each side at the rear of the trailer.

The S2-S4 design configuration was further subjected to sensitivity analysis to determine at which outside temperatures supplemental cooling and heating systems will be needed to maintain acceptable thermal condition inside the trailer. Ultimately, summer simulations on S2-S4 configuration suggested the need for a cooling system, i.e., a water sprinkling system when the outside temperature rises above 22°C. Figure 2.3A shows comparison of interior temperature at different summer outdoor temperatures. On the other hand, winter sensitivity analysis suggested operation of a heating unit to pre-heat incoming air before it passes through the air filters when outdoor temperature is lower than -10°C. Figure 2.3B shows predicted temperature profiles at three different winter outdoor temperatures.



(A)



(B)

Figure 2.3. Contours of predicted temperature inside the trailer at three different summer (A) and winter (B) outdoor temperatures from the sensitivity analysis. Figure obtained from *Predicala et al., 2017.*

2.6 Summary

A few of the emerging and re-emerging economically significant swine diseases, particularly in North America, have been proven capable of airborne transmission up to long distances. The swine industry in the past two decades responded to this biosecurity risk posed by long-distance

transport of swine pathogens by implementation of air filtration systems in barns, on top of the heightened biosecurity protocols that were already in place. The installed air filtration systems varied in terms of materials used, costs and resultant efficacy in protecting swine herds from airborne pathogens.

Transportation being an inevitable process in livestock production subjects animals to unfamiliar surroundings, unfavorable or often times extreme environmental conditions making transportation an overall stressful period in the life of the animals. Several studies that investigated the impact of transportation on animal health, welfare, meat quality, and productivity in terms of minimizing dead or down on arrivals, showed there is a need to improve the current design of commercial livestock vehicles. Initial investigations on operating mechanical ventilation on livestock compartments, although limited, has showed promise in improving environmental conditions for animals in transport.

2.7 Knowledge gap

Previous studies have proven the efficacy of air filtration in reducing risk of airborne transmission of economically significant swine pathogens in swine barns. Also, findings from studies which investigated the thermal environment inside a livestock trailer and its impact on animal welfare during the transport process suggested re-design and interventions on the existing livestock transport vehicles. This current study aimed to fill the following knowledge gaps:

1. performance of an air-filtration system installed on an enclosed swine transport trailer in preventing entry of pathogen-laden particles into the trailer through aerosols produced using a model or surrogate virus;
2. a thorough understanding of the swine micro-environment, both thermal and air quality, inside a trailer provided with mechanical ventilation;
3. ventilation rates required for a mechanical ventilation system to maintain desirable environmental condition inside the trailer during typical cold weather conditions in Canada, particularly in the province of Saskatchewan;
4. costs associated with installation of a filtration system and operation of a mechanical ventilation system on a swine transport trailer; and
5. design strengths and weaknesses of a developed prototype trailer to determine re-design alternatives and supplemental equipment for improved performance.

Findings from the comprehensive evaluation of the performance, potential and limitations of the developed prototype air-filtered trailer will contribute to the ultimate goal of helping eliminate the biosecurity gap in the swine industry during animal transport and at the same time address some transport-related animal welfare concerns.

2.8 Research objectives

This graduate research was part of a four-phase project funded by the Saskatchewan Ministry of Agriculture and Agrivita Canada with the main goal of developing a new and improved design for animal transport trailer that will facilitate control of airborne pathogen contamination and improve operational efficiencies. The specific objectives of this thesis research were:

1. evaluate the overall effectiveness of the air-filtered trailer in preventing airborne pathogen introduction to swine being transported;
2. assess trailer's capacity to provide stable and acceptable thermal environment and air quality inside the trailer during transport (i.e., from loading to unloading);
3. characterize cost and economic feasibility of the new trailer design for commercial swine production; and
4. develop recommendations for design optimization of the air-filtered trailer and accompanying management practices to prevent airborne pathogen entry and to optimize environmental conditions inside the trailer during transport.

CHAPTER 3 MATERIALS AND METHODS

3.1 Overview of the research plan

The overall approach to this study is presented in Figure 3.1. The prototype air-filtered trailer was assembled and evaluation of its performance was conducted in two parts. The stationary test was aimed to test the capacity of the trailer's air filtration system to prevent airborne introduction of pathogens inside the animal compartment. The test used a model virus to simulate airborne swine pathogens and was carried out inside Hardy Laboratory at the College of Engineering, University of Saskatchewan. The road test, on the other hand, was intended to assess the resultant environmental condition inside the air-filtered trailer during an actual journey with the trailer loaded with pigs to capacity. A preliminary cost analysis was done based on actual costs incurred in the assembly and evaluation of the prototype trailer. Recommendations for design optimization were formulated from data and observations obtained from the tests.

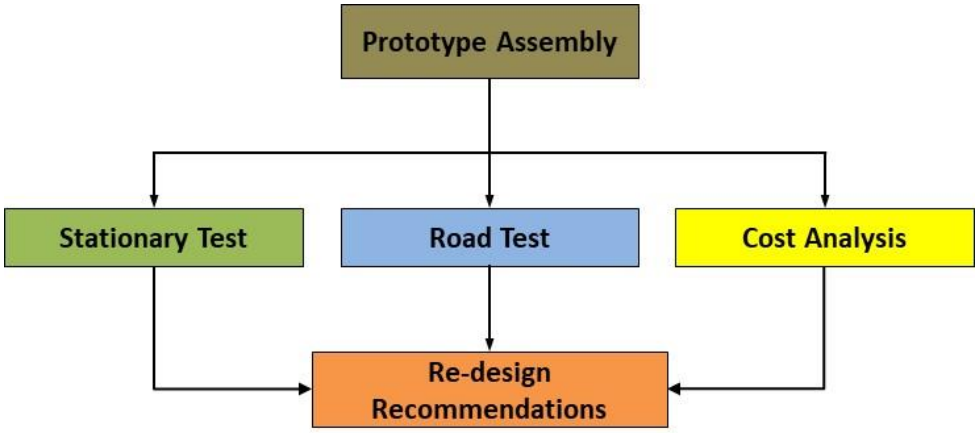


Figure 3.1. Overall framework of the study.

3.2 Description of the prototype air-filtered trailer

The assembled prototype trailer was made up of two main compartments, the front compartment and the animal compartment, both installed on top of a flatbed trailer. The following provides description of the two compartments.

3.2.1 Front compartment

The front compartment held components of the trailer air filtration and ventilation systems. It was made out of a metal 10' × 8' × 7.5' (l × w × h) storage container. A 10-kW, single-phase generator set (PowerLine™ Model KS1000-T4, Frontier Power Products, AB, Canada) was installed in the front section of the compartment. Two 2' × 6.25' (w × h) openings on both sides of the compartment secured using steel mesh and detachable pre-filters served as main air inlets for the livestock trailer. Air inlet on the driver side also served as access door to the front compartment. The air filter wall, sealed on all sides, held 6 filter sets each composed of a 24 × 24 × 1 MERV 8 pre-filter (30/30®, Camfil Farr, AB, Canada) and a 24 × 24 × 12 MERV 16 filter (Durafil® ES, Camfil Farr, AB, Canada). The MERV 8 pre-filter according to manufacturer was made from "proprietary blend of fibers" with a mechanical principle of particle capture. Its radial pleat design was maintained by molded wire grids. The MERV 16 filter, on the other hand, was made from microfine glass fibers to form mini-pleats assembled into multiple V-banks. Table 3.1 summarizes characteristics of the filter media used. Two 18-inch diameter axial fans, each powered by 2 HP, 3-phase electric motor (Sukup, Sheffield, IA USA) were installed at the downstream side of the filters to pull fresh air through the air filter sets and onto the animal compartment at a controlled flow rate. A commercially available centralized electronic control system, Maximus System (Maximus Systems, Saint-Bruno-de-Montarville, QC, Canada) was utilized to control the mechanical ventilation system of the prototype trailer. The control system came with two variable frequency drives, VFD (Leeson SM2 Series Flux Vector, Regal Beloit Canada, ON, Canada). Schematic diagram of the front compartment is shown in Figure 3.2.

Table 3.1. Technical information on the filter media used.

Characteristics*	Pre-filter	Secondary filter
Description	Pleated panel filter	V-style filter
Filter material	Blend of cotton and synthetic fiber	Microfine glassfiber in minipleat design
Dimensions, inch	23.5 × 23.5 × 0.88	23.38 × 23.38 × 11.50
Media surface area, ft^2	9.8	200
Initial resistance at capacity	0.23 inch w.g. at 1400 cfm	0.60 inch w.g. at 2000 cfm
ASHRAE Standard 52.2 - 2007 rating	MERV 8	MERV 16

*Information adapted from Camfil USA, 2014.

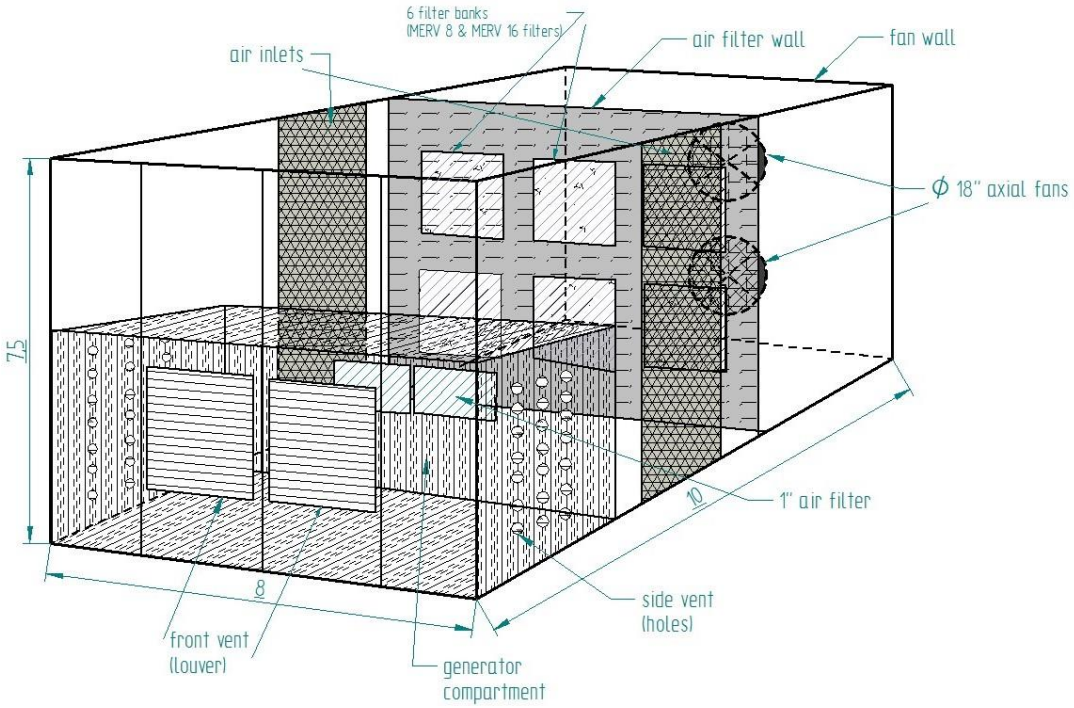


Figure 3.2. Schematic diagram of the set-up of components of the trailer air filtration and ventilation system in the front compartment.

3.2.2 Animal compartment

The animal compartment is a 20’ × 7.25’ × 7’ (l × w × h) box of aluminium 5754 H111 construction (Figure 3.3). It has solid walls, in contrast to the walls of conventional livestock trailers where side vents are present throughout the entire length of the trailer. It has two decks (top and bottom) each divided into two pens (front and rear) by a gate. Both top and bottom decks are 3’5” in height. The middle portion of the upper deck floor is hinged and can be lifted open to allow easier loading, unloading or other human activities (i.e., trailer cleaning, washing, inspection) in the bottom deck. Similarly, the middle portion of the trailer roof is hinged for the same activities in the upper deck. Additionally, air shocks are employed in these hinged floor and roof for reduced effort in lifting open and added safety in closing. Figure 3.3 showcases the features of the livestock container that was custom-built for this study (Castañé Group, Barcelona, Spain).

The ventilation fans were installed in the front compartment such that each fan supplied air to the animal compartment through openings located at the top center of the front wall of the top and bottom decks of the animal compartment. A frame with steel bars were used to prevent animals from contact with the fan blades (Figure 3.3A). On the other hand, exhaust air openings are located

Figure 3.3. Photos of the animal compartment showing (A) its lower and upper decks, (B) hinged roof, (C) gate that partitions one deck into two pens, (D) air exhaust damper, (E) hydraulic lift gate, (F) hydraulic lift controller and (G) compartment exterior.

on both sides at the top rear of each deck. To prevent unfiltered air from entering the animal compartment through these openings, 2.5’ × 1’ (w × h) backdraft dampers (Kehfab, Steinbach, MB) were installed on the exhaust openings and a duct frame attached on the outside of each opening to partially deflect wind from directly impacting the backdraft dampers during travel (Figure 3.3D).

To address animal handling and welfare issues faced with use of ramps in conventional livestock trailers, a 1,000-kg capacity hydraulic lift gate was added to the prototype trailer (Figure 3.3E). Its control system composed of a hydraulic motor powered by two automotive batteries and a push-button type remote as shown in Figure 3.3F.

3.3 Stationary test

3.3.1 Preparation of the test virus

In this test, bacterial virus or bacteriophage Phi X174 (ATCC 13706-B1) together with its host *Escherichia coli* (ATCC 13706) was used as surrogate for common viral swine pathogens. Phage Phi X174 is tailless, non- enveloped, 25 - 27 nm in capsid size, and contains single-stranded DNA (ssDNA) as its genomic material. The bacteriophage and its host were obtained from the American Type Culture Collection (www.atcc.org). Phage Phi X174 was propagated on liquid culture of *E. coli* and then aerobically incubated at 37°C with shaking at 160 rpm for 24 hours. Centrifugation at 2,000 × g for 10 minutes followed to remove the lysed cells and debris from the phage lysate. Finally, filtration of the phage lysate was carried out using 0.22 μm vacuum filtration unit and then stored at -80°C until use. Preparation and storage of the amplified phage stock (2.78×10^{10} ssDNA copies/ml) were done by trained personnel at the Microbiology Laboratory of Western College of Veterinary Medicine (WCVM), University of Saskatchewan. This method of viral stock preparation was adopted from Broyles et al. (2002).

3.3.2 Aerosol generation and viral load sampling

On the day of testing, a nebulization solution composed of 1 mL phage lysate diluted in 39 mL ultra pure water (Barnstead Nanopure Diamond, Barnstead Thermolyne Corp., IA, USA) was prepared. A cold fog mister (Hurricane ULV/mister, Curtis Dyna-Fog Ltd. Westfield, IN, USA) was then used to generate aerosol at an average liquid use rate of 37.5 mL/min. Aerosol produced was directed into a 2’ × 2’ × 1.5’ (length × width × depth) chamber made of cardboard lined with

aluminum foil installed upstream of the air filter set directly in front of the bottom deck fan. The same foil-lined cardboard material was used to create a duct that connects the downstream side of the air filter set to the inlet side of the bottom deck fan, to ensure that all air entering into the chamber upstream of the filter set passes through the filter and all the way through the fan. Downstream of the fan and inside the animal compartment, a 2' × 2' chamber that was 4' in depth received the air flow from the fan. This testing setup was used to ensure maximum capture of the aerosols that passed through the filter set being tested. A smoke test was done prior to testing to locate and seal leaks all over the testing setup. For the entire duration of the test, the ventilation system was run at 10% of fan capacity with estimated ventilation flow rate of 2000 L/min for the bottom deck ventilation fan only. Viral load samplings were done at the bottom deck of the livestock container only. Each filter set change represented a replicate. Figure 3.4 and Figure 3.5 show the testing set-up.

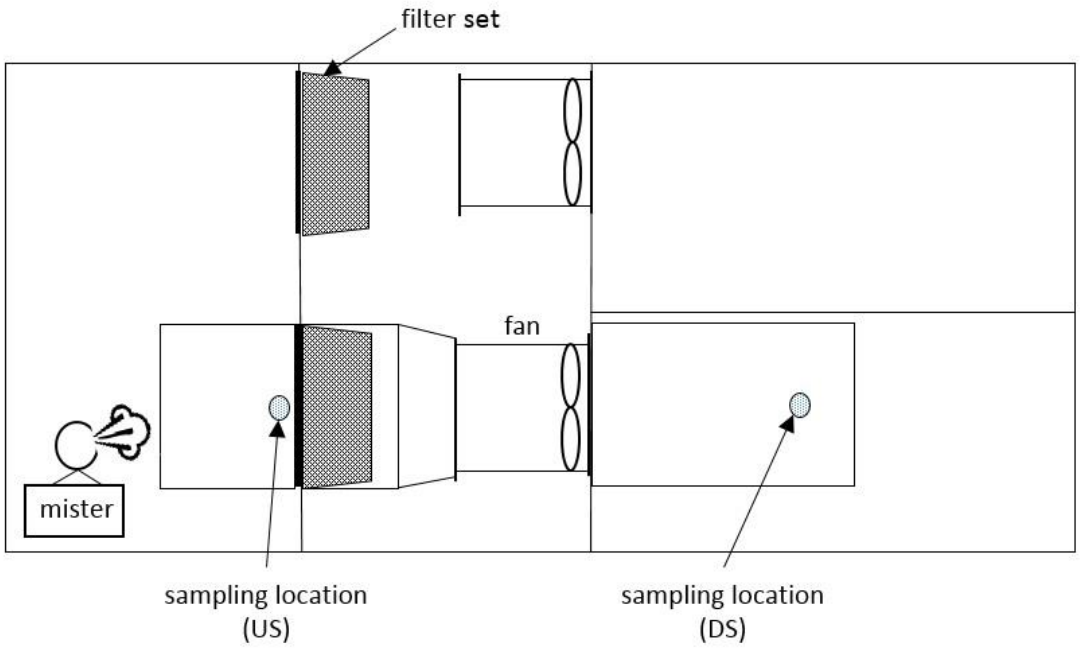


Figure 3.4. Diagram of the testing setup during the stationary test. US and DS stand for upstream and downstream, respectively.

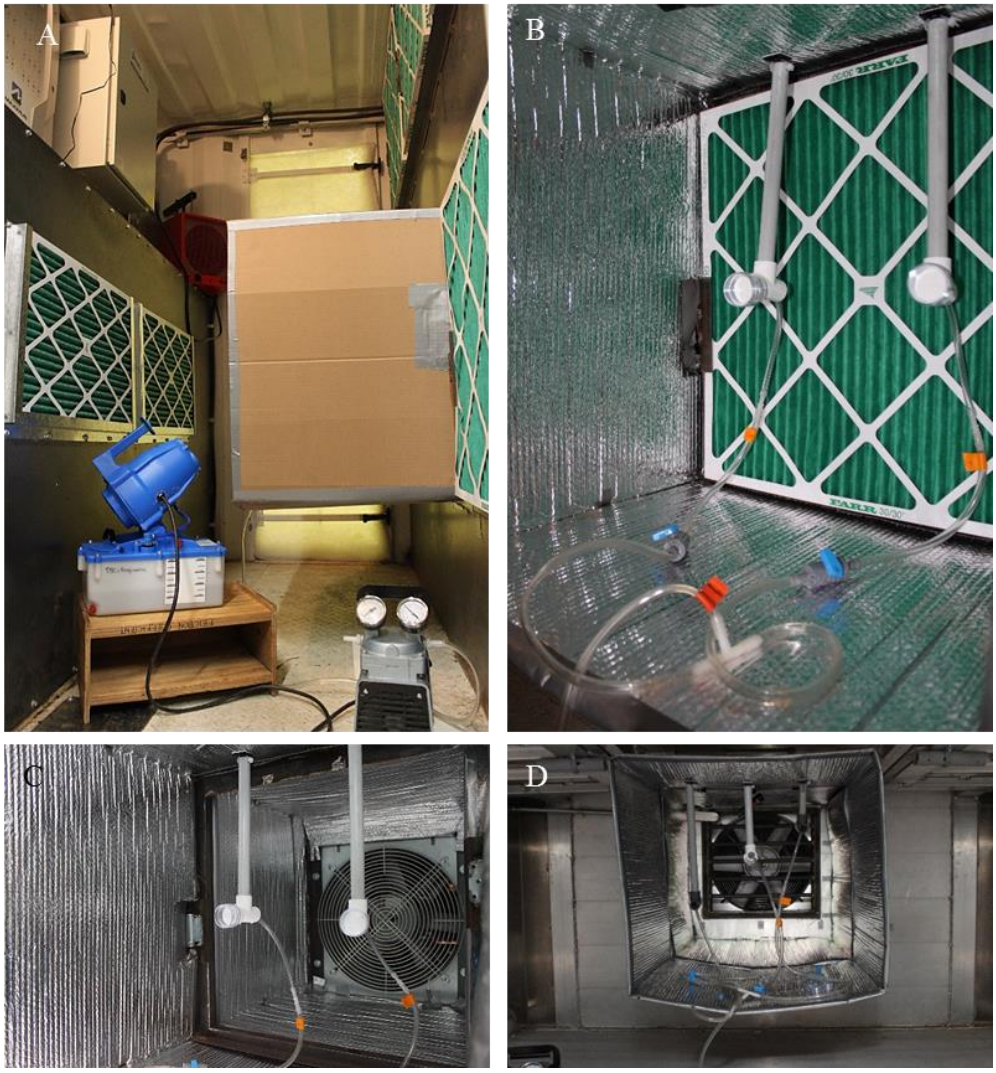


Figure 3.5. Photos of the testing setup. (A) shows exterior of the chamber upstream of the air filter and the nebulizer used. (B) is inside the upstream chamber with two cassette samplers and air filter set. (C) shows the duct from upstream (air filter set removed) to downstream of the air filter set connecting to the bottom deck fan. (D) shows the 4' depth chamber downstream in the animal compartment with three cassette samplers.

Polycarbonate filters (PC) (SKC Inc., Eighty Four, PA) with 0.4 μm porosity and 37 mm in diameter mounted on cellulose support pads placed in 37 mm clear styrene 3-piece cassettes (Sureseal, SKC Inc.) were used to monitor phage concentration in aerosols upstream and downstream of the air filter set and fan assembly. For every sampling repetition, two cassette samplers were placed in the upstream location and three samplers downstream in the animal compartment 3' from the ventilation fan grill (Figure 3.5). Aerosolization and sample collection duration was 10 minutes. Also, approximately 5-minute downtime in-between sampling was needed for replacing cassette samplers and to allow the phage concentration in the upstream and

downstream compartments to dissipate prior to starting succeeding repetition. The open-face filter cassettes were connected to Gast air sampling pumps (Gast Manufacturing, Benton Harbor, IM, USA), model DOA-P704-AA for upstream samplers and DAA-V715A-EB for downstream samplers. Sampling flow rates for each cassette samplers were determined following isokinetic sampling principles. Isokinetic sampling was necessary to prevent divergence of flowlines at the sampler inlets and consequently avoid over- or under-sampling the particle concentration compared to the main air stream (Wilcox, 1956). Using VelociCalc® Air Velocity Meter 9545-A (TSI, Shoreview, MN), the average air speed at different sampling locations were determined and the sampling flowrates for each sampler were adjusted to match the average air speeds determined. PVC ball valves were used to adjust flow rate for each sampler and flow calibration was done using Bios DryCal® DC-Lite Model DCL-M (Bios International, Butler, NJ, USA). Average sampling flowrates for upstream samplers were 1.823 and 1.873 L/min while for downstream samplers were 9.350, 5.148, and 3.520 L/min.

In this test, each air filter set change represented a replication. Consequently, a total of 4 replications each consisting of six sets of 10-min upstream-downstream sampling repetitions with five measurement points for each repetition were conducted. Moreover, to validate integrity of the sampling method and set-up, 2 positive control tests (aerosolized phage solution with air filter set removed) and 2 negative control tests (distilled water was aerosolized with air filter set on) were carried out. For each control test, five cassette samplers were set-up as previously described. Thus, a total of 140 polycarbonate filter samples were collected. After each test, these filter samples were stored individually in 15 ml conical centrifuge tubes at -80°C for subsequent qPCR analysis.

3.3.3 Extraction of viral particles from filters and qPCR

Extraction of total genomic DNA from filter samples was done using methods described by Anderson-Glenna et al. (2008) with modifications. From storage, each filter paper sample was cut into strips on petri dish and soaked in 5 ml phosphate buffered saline. Elution of genomic DNA from filter strips was done as follows: 556 µl of 10% sodium dodecyl sulfate was added and then incubated at 65ºC for 10 minutes; then 32 µl of RNase A (DNase and Protease-free, 10 mg /ml, Thermo Fisher Scientific, MA, USA) was added and incubated at 37ºC for 30 minutes; and finally 63 µl of Proteinase K (Thermo Fisher Scientific, MA, USA) was added and then incubated at 55ºC for 45 minutes. From this, 1 ml of lysate was transferred to 2 ml microcentrifuge tube. A three-

step centrifugation process followed. Centrifugation at 13,100 rpm for 3 minutes was done after adding 1 ml phenol:chloroform:isoamyl alcohol (25:24:1) (Sigma-Aldrich, MO, USA). The top aqueous layer was then transferred to a clean 2 ml microcentrifuge tube and 350 µl of chloroform:isoamyl alcohol (24:1) (Sigma-Aldrich, MO, USA) was added and centrifuged at 13,100 rpm for 3 minutes. Again, the top aqueous layer was transferred to 1.5 ml microcentrifuge tube, 1/10th volume of 5 M sodium chloride (G-Biosciences, MO, USA) and 2 volumes of 95% ethyl alcohol were added, and then kept at -20 ºC for 30 minutes before centrifugation at 16,100 rpm for 10 minutes at 4 ºC. The supernatant was discarded and the pellet was washed with 70% ethyl alcohol to remove any residue salts and centrifuged at 13,100 rpm for 3 minutes to remove remaining supernatant. Finally, the tubes were air-dried and the DNA pellet was resuspended in 50 µl Tris-EDTA buffer (TE buffer, 1x, Promega Corp., WI, USA) and kept overnight at 4ºC.

3.3.4 Quantification of phage particles by qPCR

The primers used for Phi X174 in this study were designed using Primer3Plus version 0.4.0 software. The forward primer used was 5'–ATCCCAATGCTTTGCGTGAC-3' and the reverse primer was 5'-TGGAAATGAAGACGGCCATT-3'.

Real-time quantitative PCR (qPCR) was done using a ready-to-use master mix optimized for dye-based qPCR, 2x iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, CA, USA). The PCR reaction mix consisted of 25 µl of the master mix, 2 µl each of the forward and reverse primers, 17 µl of ultra pure water and 4 µl of the extracted total genomic DNA from the polycarbonate filter for a final reaction volume of 50 µl for each filter sample. qPCR reactions were run on a plate containing duplicate assays of no-template control, the unknown samples, and a standard composed of target-containing plasmids at concentrations ranging from 10^0 to 10^7 copies/reaction. The PCR program involved incubation at 95ºC for 3 minutes, followed by 40 cycles of 95ºC for 15 seconds, 55ºC for 15 seconds, and 72ºC for 15 seconds and a final extension at 95ºC for 10 seconds. A melt curve was then generated starting at 55ºC to 95ºC at 0.5ºC increments and holding post-incubation at 95ºC. Fluorescence measurements occurred every cycle at the end of annealing step and during melting curve data collection. All analysis were performed using a thermocycler (CFX Connect™ Real-Time PCR Detection System, BIO-RAD Laboratories, Inc., CA, USA).

Bacteriophage concentration in the nebulization solution used for testing was determined by heating in water bath at 96ºC for 10 minutes and qPCR was performed using the same amplification program used for filter samples.

Extraction of viral particles from filter samples and qPCR were carried out by trained personnel at WCVM, University of Saskatchewan, using their laboratory facilities and equipment.

3.3.5 Calculation of filtration efficiency

Finally, filtration efficiency in terms of viral load was estimated using Equation 3.1 (Peska and Lebkowska, 2012; Ardkapan et al., 2014):

$$\eta_{i,filter} = \frac{L_u - L_d}{L_u} x\ 100 \tag{3.1}$$

where $\eta_{i,filter}$ is the air filter efficiency (%) and L_u and L_d are the average viral loads (ssDNA copies/L of air) upstream and downstream of the air filtration system, respectively. L_u and L_d were derived from:

$$L = \frac{C \times v}{f \times t} \tag{3.2}$$

where C (ssDNA copies/mL) is the viral or phage concentration of the sample, v (mL) is the sample volume, f (L/min) is the sampling flow rate for each cassette sampler and t (min) is the sampling duration (Turgeon et al., 2014).

3.4 Road test

Two monitoring trips from a pig farm in Saskatoon, SK to an abattoir in Moose Jaw, SK with market pigs loaded inside the trailer were done under winter conditions (December 1 and 14, 2017). The average live weight of the market pigs, stocking density, time of start of loading, travel interruptions on the road and time until end of unloading in the abattoir were recorded.

The route used during the monitoring trips, shown in Figure A.1 (Appendix A), was chosen to achieve travel time of not less than 5 hours excluding time allotted for loading, wait time in the yard of the abattoir and unloading.

The mechanical ventilation system was turned on before start of loading and was kept operating until end of unloading. During the trip, real-time access to the ventilation system controller was done using a tablet through wifi connection to the Maximus System via an air router.

3.4.1 Data collection

The instrumentation system applied was to ensure continuous data logging for the entire duration of each monitoring trip. Protection of sensors and data loggers from potential physical damage by animals as well as being unobtrusive during loading and unloading were considered in the design. Figure 3.6 shows the distribution of sensors and data loggers inside the trailer during the second monitoring trip. More monitoring devices were installed during the second trip and will be indicated during presentation of results in subsequent chapter. Moreover, the monitoring devices were installed at the ceiling of each deck approximately 1 m (≈ 40 in) above the floors which was approximately 0.3 m above the pig level. This was done to ensure the devices were kept from animal damage. It was assumed that due to the relatively small enclosed space and the close proximity of the actual sensor location (i.e., within 0.3 m only), then the measured parameters were adequately representative of the conditions at the animal zone.

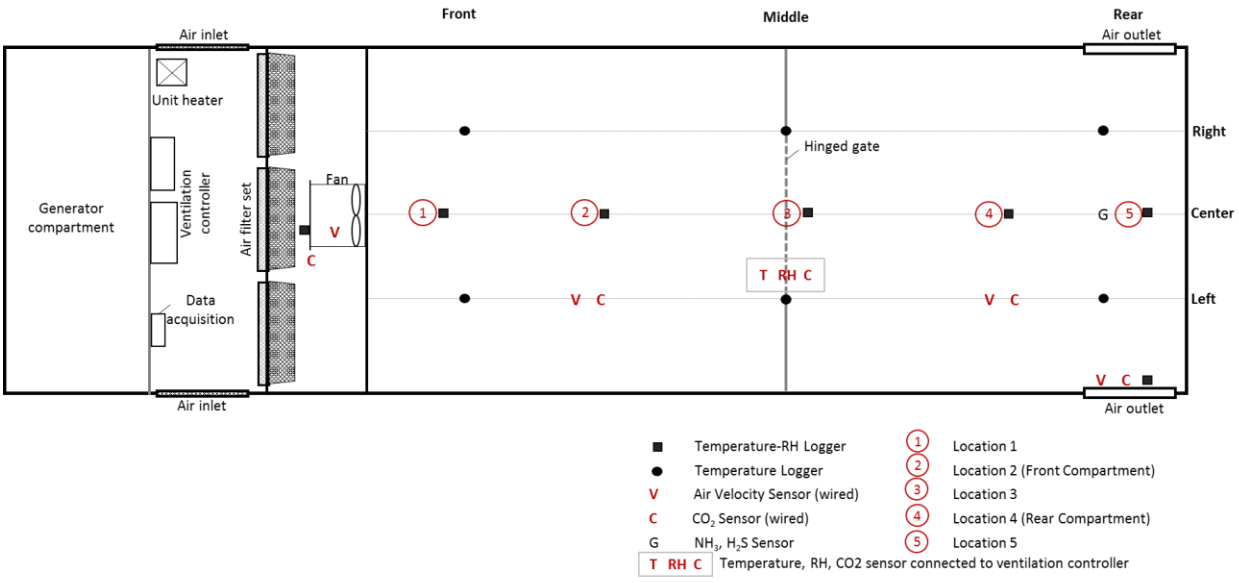


Figure 3.6. Trailer schematic diagram showing the plan view of locations of sensors and data loggers used to measure temperature, relative humidity, air velocity, CO_2 and other gases (NH_3 and H_2S) levels inside the trailer. Similar layout was followed for both top and bottom decks of the animal compartment.

In subsequent sections, it should be noted that the interior of the livestock container was spatially divided into five main zones: Location 1 represents area close to the ventilation fans; Location 2 for center of the front pen; Location 3 for center of the entire livestock container length; Location 4 for center of the rear pen; and, Location 5 for area close to the air outlets found at the

rear side of the trailer. This placement of measuring devices was used for both upper and bottom decks of the prototype trailer. Front, Middle and Rear and Left, Center and Right will be used in subsequent chapters to refer to trailer locations as depicted in the schematic diagram (Figure 3.6). Also noteworthy is that supplemental heating was installed and operated only during the 2nd monitoring trip, after initial processing of data collected in the 1st trip showed below 10°C temperature at some locations inside the animal compartment despite outside temperature not lower than -10°C during monitoring trip #1. Due to space constraints within the front compartment, only a single supplemental unit heater was installed at the top right corner in the front compartment.

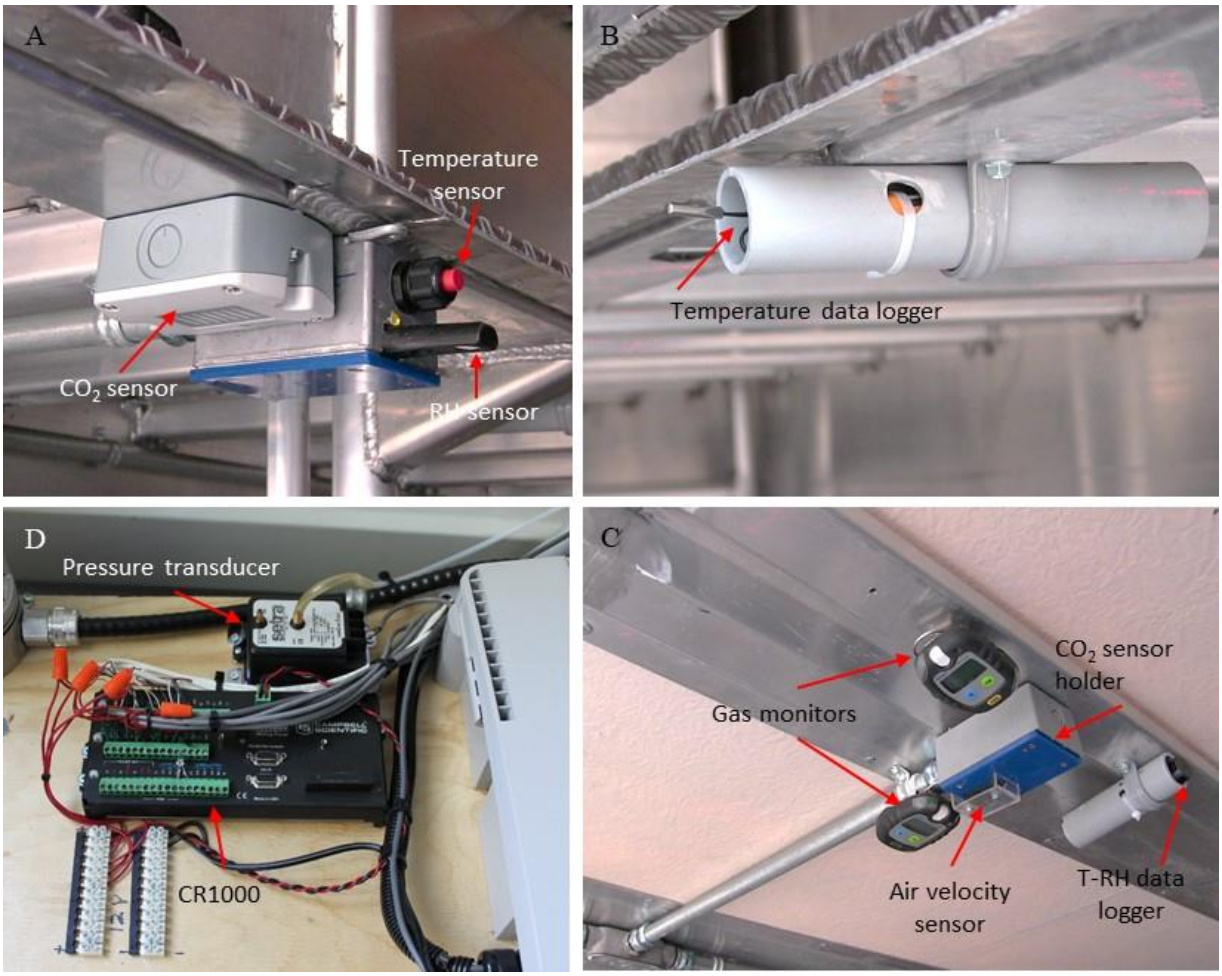


Figure 3.7. Sensors and standalone data loggers used during the monitoring trips. (A) CO_2, temperature and RH sensors that came with the ventilation controller system (Maximus Systems, Maximus) installed at the bottom deck used to control the ventilation system. (B) Thermistor-type temperature data logger protected by a PVC pipe. (C) Gas monitors with housing and holder for CO_2 and velocity sensors, and temperature-RH data logger. (D) Datalogger for the wired sensors for velocity, carbon dioxide and differential pressure and pressure transducer.

3.4.1.1 *Temperature and humidity*

Temperature and relative humidity inside the trailer were logged every 30 seconds using OM-EL-USB-TP-LCD and OM EL-USB-2 standalone data loggers (Omega Environmental, Laval, QC, Canada). OM-EL-USB-TP-LCD came with a thermistor probe and reads temperature ranging from -40 to 125°C at an absolute accuracy of ±0.1°C. OM EL-USB-2, on the other hand, is an RH-temperature-dew point data logger that operates from -35 to 80°C with accuracy of ±0.5°C for temperature and 0 to 100% with accuracy of ±3.5% for RH ranging 20% to 80%. A total of 8 units during the December 1 trip and 12 units during the December 14 trip of OM-EL-USB-TP-LCD data loggers were used to measure periphery temperatures while 6 and 10 units during the first and second trip, respectively, of OM EL-USB-2 recorded temperature and RH along the center of each deck and another 4 units for inlet and outlet temperature and RH monitoring. Additional protection was provided by data logger holders made of PVC pipes with holes for free air movement around the data loggers. Figure 3.7B and C show the two data loggers in their protective housing.

3.4.1.2 *CO_2, air speed and room static pressure*

Concentration of carbon dioxide (CO_2) during the monitoring trips were measured every 30 seconds using SE-0018 sensors (CO2Meter.com, Ormond Beach, Florida). The CO_2 sensor has a total detection range of 0 to 10,000 ppm (0 to 5,000 ppm within specifications) and accuracy of ± 30 ppm ± 3% of measured value within specifications. A total of seven CO_2 sensors were installed: one placed close to the center of each of the four pens of the trailer, one measured inlet concentration (representative for both top and bottom decks) and two for outlet concentration (one for each deck). On the other hand, every 15 seconds measurement of air speed at eight locations inside the trailer were made using D6F-W10A1 air speed sensors (Omron, Japan). Specification range for the air flow sensor is 0 to 10.0 m/s with accuracy of ±6% of full scale reading. Air speed sensors were distributed similar to CO_2 sensors except that individual air flow sensors were installed for each deck.

The CO_2 and air velocity sensors were mounted together on specially designed holders (Figure 3.7C). All wiring to connect the sensors to the data acquisition system were run through metal conduits and routed through junction boxes for protection from damage. Additionally, a pressure transducer (Setra 265, Setra, Boxborough, MA) was installed to measure static pressure

inside the trailer every 30 seconds. Measurement range for the pressure transducer is 0 to 1 inch WC and standard accuracy is ±1% of full scale reading. Data for these three parameters were logged continuously using a 16-port CR1000 data logger (Campbell Scientific Canada, Edmonton, AB) (Figure 3.7D). Wires with custom-fit lengths connected the sensors to the CR1000.

3.4.1.3 NH_3 and H_2S

Hydrogen sulfide (H_2S) and ammonia (NH_3) level monitoring were done using Dräger Pac® 7000 (Draeger Safety Canada, Ltd, Mississauga, Ontario). Measuring ranges for the gas detection devices are 0 ppm to 200 ppm for H_2S and 0 ppm to 300 ppm for NH_3. Logging interval was one minute for both gas monitors.

3.4.2 Ventilation effectiveness

Ventilation effectiveness was assessed based on the heat removal effectiveness (HRE) and contaminant (CO_2) removal effectiveness (CRE) at the animal-occupied zone (AOZ) defined as follows (Liddament, 1993 as cited by van Wagenberg and Smolders, 2002):

$$HRE = \frac{T_{outlet} - T_{inlet}}{T_p - T_{inlet}} \tag{3.3}$$

$$CRE = \frac{C_{outlet} - C_{inlet}}{C_p - C_{inlet}} \tag{3.4}$$

where HRE and CRE are the dimensionless heat and contaminant removal effectiveness at point *p*; and, *T* and *C* are temperatures (°C) and CO_2 concentrations (ppm), respectively. Subscripts *outlet*, *inlet* and *p* refer to exhaust, inlet and arbitrary point conditions, respectively. Average HRE and CRE values were computed for the top deck and for the bottom deck of the trailer.

3.5 Data processing and analysis

3.5.1 Data processing

3.5.1.1 *Viral concentration from qPCR*

Bacteriophage Phi X174 concentration (in genome copies/filter) was determined from filter samplers and analyzed by qPCR. Raw data from qPCR were categorized into three: *detected and quantified* (DQ), *detected not quantifiable* (DNQ) and *not detected* (ND). DQ qPCR values had quantitation cycle (Cq) or threshold cutting values lower than or equal to the highest Cq of the standard curve determined for every qPCR run and starting quantities (SQ, in genome copies/2

µl). DNQ data had SQ values but the Cq values were higher than the signal detection Cq of the standard curve, thus, confidence in the result provided was low. Nevertheless, for DNQ data sets, the provided SQ values were used as numerical estimate for the genome concentration of the samples they represent. ND qPCR results, however, did not provide both Cq and SQ values; hence prior to further data processing, ND qPCR results were given numerical estimate using one-half of the lowest detected limit ($SQ = 10^3$).

SQ values initially in genome copies/2 µl were converted to genome copies/L of air by applying the sampling flowrates, *f*, and sampling durations, *t*, used during stationary test on Equation 3.2.

3.5.1.2 *Environmental data from monitoring trips*

Preliminary processing of environmental data obtained from the road test involved manually segregating raw data based on timing of each transport period: *loading*, *main transport period* which is further subdivided into *early stage of trip* and *stable transport period*, *arrival on site up to end of waiting at the plant*, and *unloading*. To focus on assessing the performance of the trailer in maintaining the desired conditions during transport, only the data during the *stable transport period* was subjected to further data filtering and analysis. This transport period occurred approximately one hour after commencement of the road trip and was manually determined by inspection of time series of temperature, CO_2 and RH levels during the monitoring trips.

A two-step data filtering method was done to remove data outliers and gross sensor errors prior to computations and statistical analysis. Filtering step 1 involved removal of data outside the measuring range specifications of the sensors and data loggers used. This step was particularly applied for CO_2 data where recorded levels during travel interruptions, although at a limited time, exceeded the 0 to 5,000 ppm measuring range (with accuracy within specifications) of the sensors. Filtering step 2 involved computation of mean, minimum, maximum and standard deviation (SD) of data sets obtained from filtering step 1, and then retaining only data points that are within its 99.7% confidence interval, i.e., mean ± 3SD. This statistical method has been widely used to remove outliers in a data set (Johnson et al., 2011; Xiong, 2013).

3.5.2 Data analysis

Processed data from the stationary test were log-transformed to meet normality and homogeneity of variance requirements prior to carrying out paired samples t-test for mean comparison between upstream and downstream phage concentrations. Means and 95% confidence intervals were used to represent viral load data. On the other hand, processed data sets for the different environmental parameters monitored were descriptively analyzed using means, standard deviations, ranges and frequencies. For comparison of mean differences of environmental parameters between the two monitoring trips and between the top and bottom decks, the respective data sets were analyzed using two-way independent student's t-test. Paired samples t-test was used to conduct within trailer deck comparisons. To compare means of the temperature, humidity ratio, CO_2 levels and air speed across all monitoring locations inside the trailer, General Linear Model - Univariate analysis was performed using SPSS (IBM SPSS Statistics, Version 24.0. Armonk, NY: IBM Corp.), with each environmental parameter analyzed separately. The model included trailer locations as fixed factor and monitoring trips as random factor. Means were separated using least significant difference (LSD) option of the general linear model of SPSS. Tests of normality and homogeneity of variances were carried out by Shapiro-Wilk and Levene's tests, respectively. Overall level of significance was defined by $p<0.05$. Processing of data and preparation of graphs were done using MS Excel while t-tests were also run in SPSS.

3.6 Cost analysis and development of design optimization recommendations

Record of actual expenditures for this project were used in carrying out a cost analysis for an air-filtered trailer with a 120-pig capacity (i.e., approximately double the size of the prototype trailer assembled). Estimation of annual operational costs were based on a 10-hr journey (pig transport) done at a maximum of two times per week, with the trailer being used 90% of the year. Various other assumptions particularly in carrying out payback period analysis are described in the subsequent chapter. Lastly, recommendations for redesign of the prototype and future work were formulated.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Filtration efficiency test (stationary test)

The aim of this phase of the study was to determine the effectiveness of the air-filtered trailer in minimizing potential exposure of livestock to pathogens during transport. For this purpose, a stationary test that quantified upstream and downstream concentration of bacterial virus Phi X174 as surrogate to common swine pathogens was conducted. No pigs were inside the trailer when the test was carried out.

4.1.1 Evaluation of the sampling set-up

In order to test the integrity of the sampling method and set-up, smoke test coupled by two 10-minute each of positive and negative control tests were conducted. Smoke test was done to ensure there was no leak around the air filter set and the testing chambers (from upstream of the filter set to downstream of the fan).

No significant difference (p = 0.341) between the upstream and downstream phage concentrations was found from the positive tests conducted (Table 4.1). The positive control test indicated that no false negative results were obtained in the four trials conducted, i.e., no viral genome detected or below qPCR detection limit, as a consequence of factors other than the relative effectiveness of the air filtration system installed. The negative control tests, on the other hand, yielded no Phi X174 genome detected on both upstream and downstream sampling locations. This means that the viral genome positive results obtained, i.e., all quantifiable genome counts, were primarily due to actual concentration of the test virus in the air captured in the sampling device and not due to contamination from uncontrolled sources.

Temperature and relative humidity during the test were 19.7 ± 1.6°C and 59.4 ± 12.1%, respectively, in the upstream chamber, and 23.9 ± 0.7°C and 38.4 ± 9.8%, respectively, in the downstream chamber. The relatively cooler thermal condition in the upstream is associated with the cooling effect of mist during aerosolization. Since the effect of the air filtration system and the

Table 4.1. Filtration efficiency determined from reduction of bacteriophage Phi X174 concentration downstream of the air filters tested, $n = 4$.

Trial*	Repetition	Nebulization solution phage concentration, copies/ml	Average bacteriophage concentration, copies/m^3 of air		Filtration efficiency, %	Mean filtration efficiency, %	Standard deviation, %
			Upstream of the filter	Downstream of the filter			
1	1	3.60E+08	2.35E+08	5.17E+06	97.8	97.9	1.3
	2		6.54E+07	1.98E+06	97.0		
	3		3.93E+08	2.31E+06	99.4		
	4		1.39E+08	2.00E+06	98.6		
	5		1.14E+08	4.63E+06	95.9		
	6		2.75E+08	4.21E+06	98.5		
2	1	3.60E+08	1.01E+08	5.05E+06	95.0	96.9	2.0
	2		1.00E+08	2.59E+06	97.4		
	3		1.53E+08	3.68E+06	97.6		
	4		8.79E+07	5.21E+06	94.1		
	5		3.86E+08	2.06E+06	99.5		
	6		1.40E+08	2.91E+06	97.9		
3	1	2.40E+08	3.70E+08	1.20E+07	96.8	95.7	4.9
	2		4.09E+07	5.82E+06	85.8		
	3		3.47E+08	7.40E+06	97.9		
	4		4.21E+08	5.52E+06	98.7		
	5		1.77E+08	3.55E+06	98.0		
	6		1.45E+08	4.39E+06	97.0		
4	1	2.40E+08	1.20E+08	1.56E+06	98.7	97.2	1.7
	2		5.71E+07	1.70E+06	97.0		
	3		1.52E+07	8.61E+05	94.4		
	4		1.83E+08	3.06E+06	98.3		
	5		5.56E+07	2.07E+06	96.3		
	6		1.25E+08	2.08E+06	98.3		
+ Control	1	3.60E+08	8.66E+07	1.64E+07	na	na	na
	2	2.40E+08	1.77E+08	1.24E+07			
- Control	1	na	ND	ND	na	na	na
	2		ND	ND			

*Each replicate trial represents one filter set, i.e. MERV 8 pre-filter and MERV 16, tested.
na – not applicable; ND – none detected by qPCR

environmental condition on the infectivity of potential airborne pathogens encountered during transport was not within the scope of the current investigation, no attempt at controlling sampling temperature and relative humidity was done. Moreover, qPCR as diagnostic analysis was deemed more appropriate for this study instead of culture-dependent tests to quantify presence of viral genome that penetrates the air filtration system evaluated. Gendron et al. (2010) and Verrault et al. (2010) in their respective studies that evaluated performances of two filter samplers in the quantification of airborne bacteriophages recovery recommended qPCR over culture-based analysis.

4.1.2 Bacterial virus concentrations and filtration efficiency

Table 4.1 summarizes the results obtained from qPCR. The average phage concentration on the nebulization solution was 3.0×10^8 ssDNA copies/ml as determined by qPCR.

Figure 4.1 shows the average concentration (in genome copies/m^3 of air) of Phi X174 upstream and downstream of the air filter sets consisting of a MERV 8 pre-filter and MERV 16 filter that were tested. Significant ($p < 0.001$, $n = 4$) reduction in bacteriophage concentration was observed between upstream and downstream of the air filter sets with mean bacteriophage concentrations of 1.8×10^8 (95% CI: 1.2×10^8 - 2.3×10^8) genome copies per m^3 of air and 3.8 x 10^6 (95% CI: $2.8 \times 10^6 – 4.8 \times 10^6$) genome copies per m^3 of air, respectively. In a study conducted by Corzo et al. (2013), measured concentration of swine influenza A virus varied at different locations: 3.20×10^5 RNA copies/m^3 of air inside the barn, 1.79×10^4 RNA copies/m^3 of air outside of barn exhaust fans and 4.65×10^3 RNA copies/m^3 of air at distances 1.5 and 2.1 km away from the infected area. In a separate study by Alonso et al. (2015), concentration of three porcine pathogens in the aerosol from experimentally infected pigs were characterized. Geometric mean of RNA copies/m^3 air sampled reached as high as 4.3×10^5 for IAV, 5.1×10^4 for PRRSV, and 3.5×10^8 for PEDV depending on particle size; hence, it can be considered that the air filter sets in this current study were challenged at relatively higher bioaerosol concentrations than the levels typically encountered in actual field conditions. Testing at bacteriophage concentrations at a higher order of magnitude than what were previously measured in above-mentioned studies was done to ensure that substantial or measurable concentrations of the test virus will be captured at the downstream of the air filtration system (breakthrough) during the positive control tests, i.e., air filter set removed while challenged with viral aerosol. A series of pre-tests prior to the final static

test gave none detected (ND) results downstream in the animal compartment during positive control tests. To ensure confidence that the sampling set-up and the analytical procedures were performing properly, the phage concentration on the nebulization solution was increased and the testing set-up was modified by using ducts to control direction of airflow upstream to downstream of the air filter set being tested. Also noticeable in Figure 4.1 is the relatively lower upstream concentration at the 20-minute exposure time. This was attributed to random factors because concentration of the test virus upstream of the air filter set was kept the same during the entire duration of the final static test. In fact, testing was carried out using only one nebulization solution, one aerosol generator liquid use rate, and one ventilation flow rate throughout.

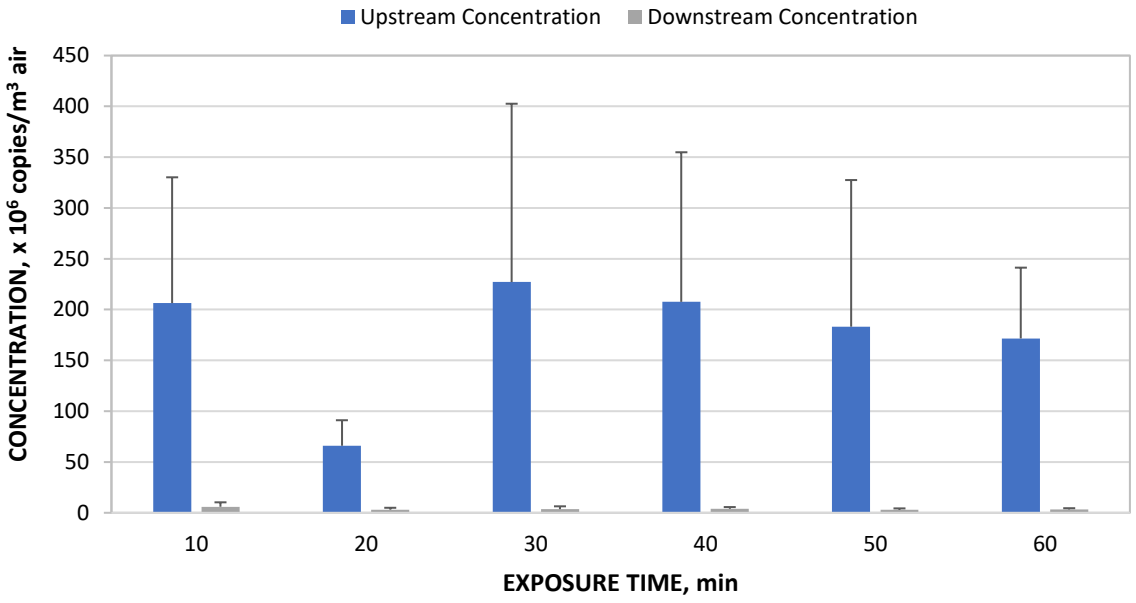


Figure 4.1. Total bacterial virus Phi X174 (in genome copies/m^3 of air) detected by qPCR. Column bars represent average concentration in four replicate trials of the surrogate virus in the air sampled using 37-mm cassettes loaded with polycarbonate filters. Error bars represent standard deviation of means.

Overall, the air filtration system installed in the trailer yielded an approximately 96.9 ± 2.8% reduction in the concentration of bacterial virus Phi X174 relative to upstream concentration as measured in the animal compartment of the trailer (Table 4.1). Although bacteriophage Phi X174 is a very small virus (Table 2.2), the air filter combination tested performed close to the expected 95% filtration efficiency for MERV 16 filters at test particle sizes ≥ 0.3 µm based on ASHRAE Standard 52.2 - 2007 testing. This may be partly due to the bigger aerosol droplets produced by the aerosol generator used in the study; manufacturer information for the Dyna-fog Hurricane

ULV/mister reports droplets produced are within the 5 to 50 µm size range. However, the Dyna-fog mister has been satisfactorily used in previous studies in generating test aerosols (Batista et al., 2008; Otake at al., 2010; Alonso et al., 2012). In a laboratory scale study conducted by Wenke et al. (2017) on four prototypes of air filter combinations for potential use in pig barns in Germany, the filter set similar to this current study (a MERV 6-8 polyester panel pre-filter and glass fiber MERV 16 secondary filter) had filtration efficiency against different pathogens including PRRSV and bovine enterovirus 1 (BEV) ranging from 95.2 to 98.6%, which are consistent with the range of values obtained in this study. Finally, the determined percent reduction in bacteriophage concentration by the installed air filtration system implies that if the system is challenged under normal field conditions (as previously determined by Corzo et al., 2013 and Alonzo et al., 2015), significant risk reduction from infection will be achieved.

Selection of the air filtration system used in the air-filtered trailer was based on the findings from a preliminary study by Predicala and Alvarado (2014) that compared performances of two filtration systems: one similar to the system used in this present study and an antimicrobial filter. Bacteriophage reduction for the antimicrobial filter was higher at 99.8% but was not significantly different from the 89.3% efficiency obtained for the other system. Cost analysis revealed it was more economical to use the pre-filter and MERV 16 filter combination. Another advantage of the chosen system was that it is suitable for both positive and negative pressure ventilation systems (Weske et al., 2017).

Unlike most air filtration system evaluation studies, this present investigation tested only one sampling condition, i.e., one bacteriophage challenge type and concentration and volume flow rates. Additionally, the aerosols generated for the test were not characterized in terms of actual particle size distribution. Thus, the filtration efficiency reported only applies for the condition under which the filtration system was tested in this study but was deemed substantial for subsequent or future case-control field studies. Ultimately, the test of the efficacy of the air filtration system of the prototype trailer should involve challenge with actual airborne swine pathogens to determine whether naïve pigs loaded in the trailer become infected or not.

4.2. Environmental condition inside the air-filtered trailer during pig transport (road test)

The aim of this portion of the study was to characterize the thermal environment and air quality inside the air-filtered trailer when pigs are inside during the transport process. Sensors and

data loggers were installed inside the pig-laden trailer during two monitoring trips conducted under mild winter condition.

4.2.1 Details of the loads of pigs for the monitoring trips

Table 4.2 summarizes the dates of the monitoring trips, number of pigs transported, space allowances used and the time for each transport event throughout the transit period.

Table 4.2. Summary of details on the loads of pigs used and the event durations for the two monitoring trips.

	Monitoring Trip #1	Monitoring Trip #2
Date of transportation	December 1, 2017	December 14, 2017
Number of pigs transported	60	61
Average weight of pigs, kg	125.5	122.8
Space allowance, m^2/115 kg pig	0.40	0.40
Event duration (hr, min)		
Loading	1 hr (4:15 am – 5:15 am)	55 min (5:12 am – 6:07 am)
Main transport period		
Early stage of trip	1 hr (5:16 am – 6:15 am)	1 hr, 12 min (6:08 am – 7:20 am)
Stable transport period	4 hr, 14 min (6:16 am – 10:30 am)	4 hr, 20 min (7:21 am – 11:40 am)
Arrival on site up to end of waiting at the plant	2 hr (10:31 am – 12:30 pm)	1 hr, 18 min (11:41 am – 12:59 pm)
Unloading	1 hr, 5 min (12:31 pm – 1:35 pm)	1 hr (1:00 pm – 2:00 pm)
Total transportation time (hr, min)	9 hr, 19 min	8 hr, 45 min

A total of 60 market-sized pigs with average weight of 125.5 ± 6.2 kg (115 – 140 kg) were loaded in the four compartments of the livestock container at 15 pigs/compartment during the first monitoring trip. For the second trip, similar group size was used with average weight of 122.8 ± 6.4 kg (114 – 140 kg). A total of 61 pigs were loaded for the second trip. Space allowance recommended by Correa (2011) as cited by Schwartzkopf-Genswein et al. (2012) for winter transport of pigs, 0.40 m^2/115 kg pig, was adopted for both trips.

Duration of different events throughout the two journeys were synonymous except that waiting period at the plant took longer during the first trip than the second trip. Moreover, journey

started earlier during the first trip (loading at 4:15 am) while second journey started at around 5:15 am. Duration of the entire transportation process was approximately nine hours for both monitoring trips.

One pig was found dead at the rear compartment of the top deck during the December 1 trip. On the same compartment, one pig was found non-ambulatory during unloading on the December 14 trip. Direct causes of the dead or down-on-arrival (DOD) pigs could not be ascertained as no surveillance cameras were installed inside the trailer during transit and no necropsy was conducted. Due to the limited number of monitoring trips conducted, the rate of DOD in this current study cannot be determined whether directly associated to the air-filtered trailer design.

4.2.2 Operation of the trailer mechanical ventilation system

The active ventilation system for the air-filtered trailer prototype was programmed as shown in Table 4.3. Ventilation flow rate was independent for each trailer deck and was primarily controlled depending on temperature measured by temperature sensors installed at the center of each deck. Minimum ventilation was set at 10% of fan capacity. At compartment temperatures greater than 16.5°C, the ventilation fans ramped up from its minimum to a maximum of 100% fan capacity corresponding to a total temperature increase of 7.5°C above the set-point of 16.5°C. This indicates that at 24°C or higher compartment temperatures detected by the trailer decks temperature sensors connected to the Maximus control system, the corresponding ventilation fan will be operating at 100%. During the second monitoring trip, RH and CO_2 compensation as stated in Table 4.3 were applied. This flexibility in ventilation control was one feature of the installed ventilation controller system.

Table 4.3. Ventilation system control program applied for the prototype air-filtered trailer during the monitoring trips.

	Set-point Temperature, °C	Ramping Temperature, °C	Minimum Ventilation, % Fan Capacity[†]
Temperature	16.5	7.5	10%
*RH compensation - 20% ramping up in ventilation from minimum of 10% as RH rises above 70% to 80%.			
*CO_2 compensation - 20% ramping up in ventilation from minimum of 10% as CO2 rises above 2500 ppm to 5000 ppm.			

*RH and CO_2 compensations were only applied during the December 14 monitoring trip.
[†]Variable frequency drives (VFD) of the ventilation fans set at 39 Hz from 60 Hz full capacity.

Minimum ventilation for the trailer was determined based on a total of 60 market pigs to be loaded in the trailer at an average weight of 130 kg/pig while maximum ventilation was computed for 50 pigs at 130 kg/pig loaded in the trailer. The reduced number of pigs assumed for maximum ventilation was based on adjusted space allowance for pig transportation during warm periods. Minimum ventilation of approximately 500 cfm (for the entire livestock container) was as dictated by moisture control at outdoor temperature as low as -35°C (Albright, 1990). Maximum ventilation, on the other hand, was computed using the recommendation from Mitchell and Kettlewell (2008) for estimation of ventilation flow rate for livestock in transit (Equation 2.1). The computed maximum ventilation was approximately 5,600 cfm for a design outdoor temperature of 25°C. Initial calibration of the installed ventilation fans (data not shown) suggested that the maximum volume flow rate could reach as high as approximately 9,200 cfm for the whole trailer. Thus, the variable frequency drives (VFD) of the mechanical ventilation control system were adjusted to 39 Hz from a full capacity of 60 Hz to ramp down fan motors' frequency. Appendix Figure A.2 gives the calibration of the two fans at the setting applied during the two monitoring trips. As shown, minimum volume flow rate at 10% of fan capacity was approximately 440 cfm while maximum ventilation at 100% fan capacity was approximately 6,700 cfm. The latter was not experienced during the two trips.

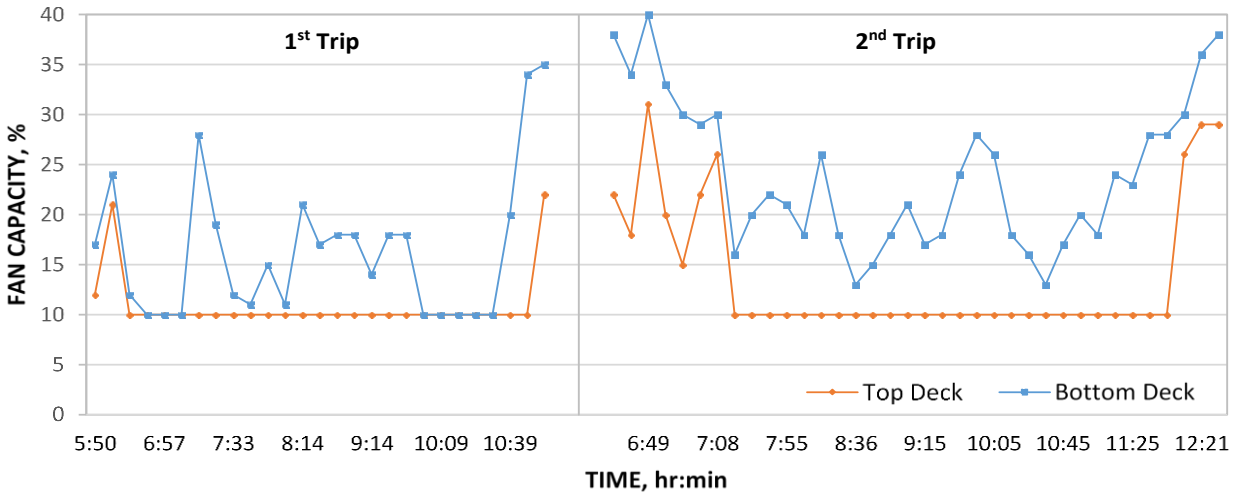


Figure 4.2. Actual ventilation schedule for the two monitoring trips.

Figure 4.2 shows the actual ventilation schedule manually recorded during the two monitoring trips. Temperature during the stable transport period at the top deck location where the sensors connected to the Maximus control system were located was significantly lower ($p < 0.05$)

compared to temperature in the corresponding bottom deck location. In fact, based on manually recorded temperatures from real-time monitoring of temperature detected by the said sensors, temperature in the top deck location did not reach the set point of 16.5°C throughout the stable transport periods of both monitoring trips. Consequently, ventilation flow rate was at the minimum (10% of fan capacity) for the entire stable transport period at the top deck. Ventilation ranges for the entire transport process were at 10% - 22% and 10% - 35% for the top and bottom decks, respectively, during the first trip. Corresponding values for the second trip were 10% - 31% and 13% - 40%. Considering only ventilation flow rates during the stable transport periods of the two trips, average ventilation flow rates for the top and bottom decks were 10 ± 0.0% (≈ 220 cfm) and 14 ± 4.9% (≈ 300 cfm) for the first trip and 10 ± 0.0% (≈ 220 cfm) and 20 ± 4.5% (≈ 415 cfm) for the second trip.

4.2.3 Temperature distribution and overall thermal conditions during transport

4.2.3.1 *Temperature time series for the entire transport*

Figure 4.3 provides the temporal variation in temperature throughout the course of monitoring trips 1 and 2. The trend in temperature for corresponding deck levels (top and bottom) were similar for both trips. Temperature for the bottom deck continually rises from the start of loading until it peaked during the early period of travel. Top deck temperature on the other hand, had an initial increase in temperature for approximately 30 minutes from start of loading, dropped for a short period and increased until peak during early period of the travel. This observation in the top deck temperature was due to the hinged middle portion of the top deck floor that was flipped open while loading pigs in the bottom deck compartments which briefly exposed the top level to heat generated in the bottom deck while it was being filled with pigs. The hinged floor was then closed after filling each pen in the bottom deck to capacity before commencing to load the top deck. This explains the momentary decline in temperature followed by gradual increase as loading progressed in the top deck until early stage of trip. Temperature from start of loading to early period of the trips ranged from -7 to 22°C and from -0.5 to 23°C for the first and second monitoring trips, respectively. Although outdoor temperature was similar for both monitoring trips, minimum interior temperature recorded for the second trip was generally higher compared to the first trip. This is attributed to the use of a unit heater installed adjacent to one of the trailer air inlets (Figure 3.6) during the December 14 trip only.

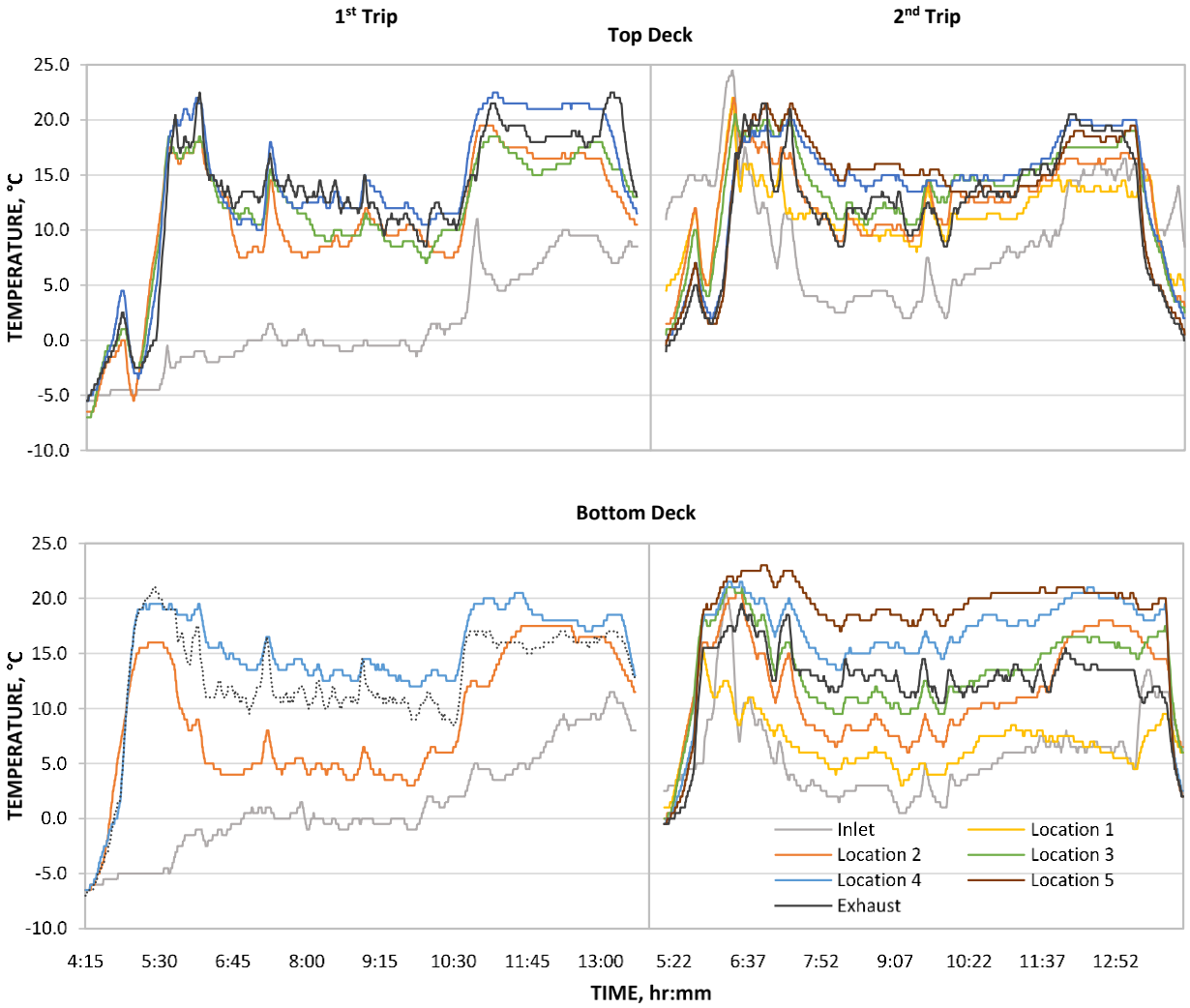


Figure 4.3. Variation in inlet, exhaust and internal trailer temperature measured at different locations approximately 1 m (≈ 40 in) above the floor along the center of the trailer top and bottom decks during the two monitoring trips. The time series represent temperature levels from start of loading in the farm to end of unloading in the plant.

Temperature for both deck started to stabilize approximately one hour after leaving the farm, except some minor peaks, such as during slow down upon entering a town center together with a short stop from 7:10 am to 7:20 am for the December 1 trip and during a stop to adjust unit heater in the front compartment between 9:30 am until 9:36 am during the December 14 trip. Consistent rise in temperature was again observed upon entering the urban center where the abattoir was located; this may be attributed to the generally slower travel speed at this stage. Compared to the temperature during the stable transport period, temperature during the waiting period at the abattoir was generally higher at 18.1 ± 2.2°C and 16.7 ± 3.9°C for trips 1 and 2, respectively. At the start

of unloading, temperature dropped first in the upper deck followed by drop in temperature in the lower deck, because the upper deck was unloaded first.

Similar observational studies on thermal condition inside swine trailers during transport corroborate most observations in this study. For instance, Kettlewell at al. (2001) and Ellis et al. (2008) found that greatest temperature extremes were observed during stationary periods such as during loading and waiting at the abattoir and during travel interruptions such as slow downs and short stops. The peaks in temperature observed at the start of loading in the current study can be explained by rise in heat production as a direct result of increased activities of the animals having been moved from the barn and into the pens inside the trailer. Periods of thermal equilibration approximately an hour after start of travel may suggest that the animals have adapted to vehicle motion. According to Kettlewell et al. (2001), the animal container structure also equilibrates during this stationary transport period. However, during travel interruptions, especially if prolonged, the thermal equilibration is disrupted as a consequence of the animals getting agitated and resulting to increase in movement. Equilibration is usually reestablished after the vehicle starts moving again. Also, from Kettlewell et al. (2001), mean heat production during their interrupted journey was higher at 1.9 ± 0.4 W/kg compared to 1.4 ± 0.5 W/kg during their uninterrupted pig transport. Although the rise in temperatures during the mentioned travel interruptions appeared favorable during cold weather condition (which is the case in our current study), this is expected to be detrimental during warm weather especially at prolonged duration.

For the entire transport period, the inlet temperature for monitoring trips 1 and 2 ranged from -6.5 to 11.5°C and 0.5 to 22°C, respectively. The significantly lower ($p<0.05$) inlet temperature for the first trip (1.5 ± 4.5°C) is again attributed to use of unit heater during the second trip (7.5 ± 4.6°C). Correspondingly, the average outlet temperatures were -7 to 22.5°C and -1 to 21.5°C. It is important to note that the data loggers for monitoring inlet temperatures for each trailer deck were located in the trailer front compartment, between the air filter wall and the fans (Figure 3.6). In this way, the measured inlet temperatures would be more representative of the air that actually entered each deck. Heat coming from the generator set and the unit heater (for the second trip) were expected to have impacted inlet temperatures recorded. Nevertheless, outdoor temperature and RH data for December 1 and 14, 2017 retrieved from Environment and Climate Change Canada (2018) at weather stations representing the towns passed on route at corresponding time during the two trips gave average temperature of -5.0°C (-9.1 to -2.1°C) for the 1st trip and -4.0°C

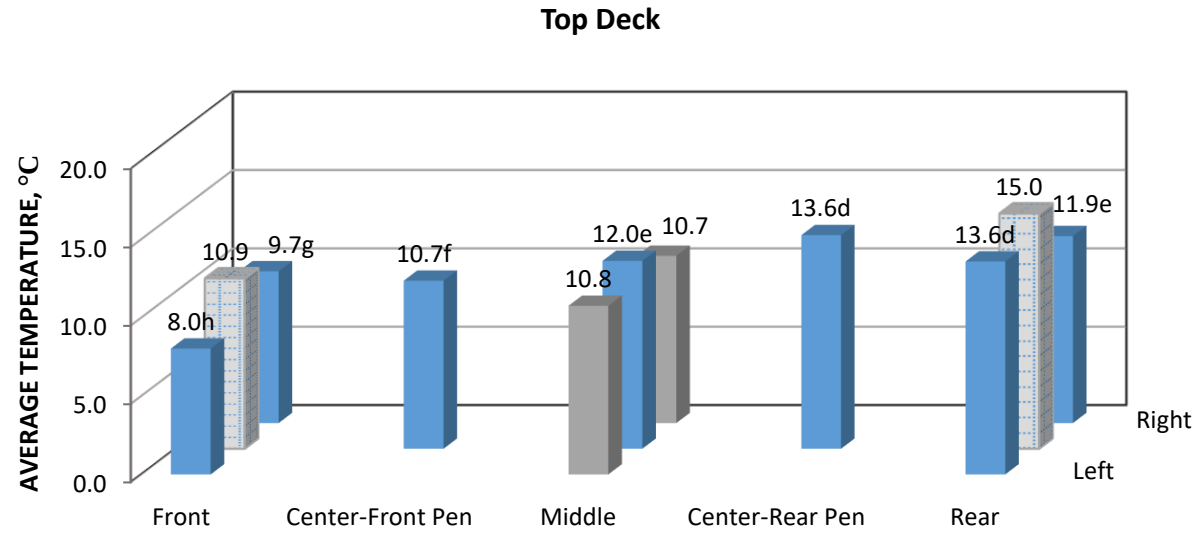
(-6.1 to -0.9°C) for the 2nd trip. Outdoor relative humidity was at 86.8% (80 to 91%) and 88.8% (80 to 92%) for the 1st and 2nd monitoring trip, respectively.

4.2.3.2 *Temperature during stable transport period*

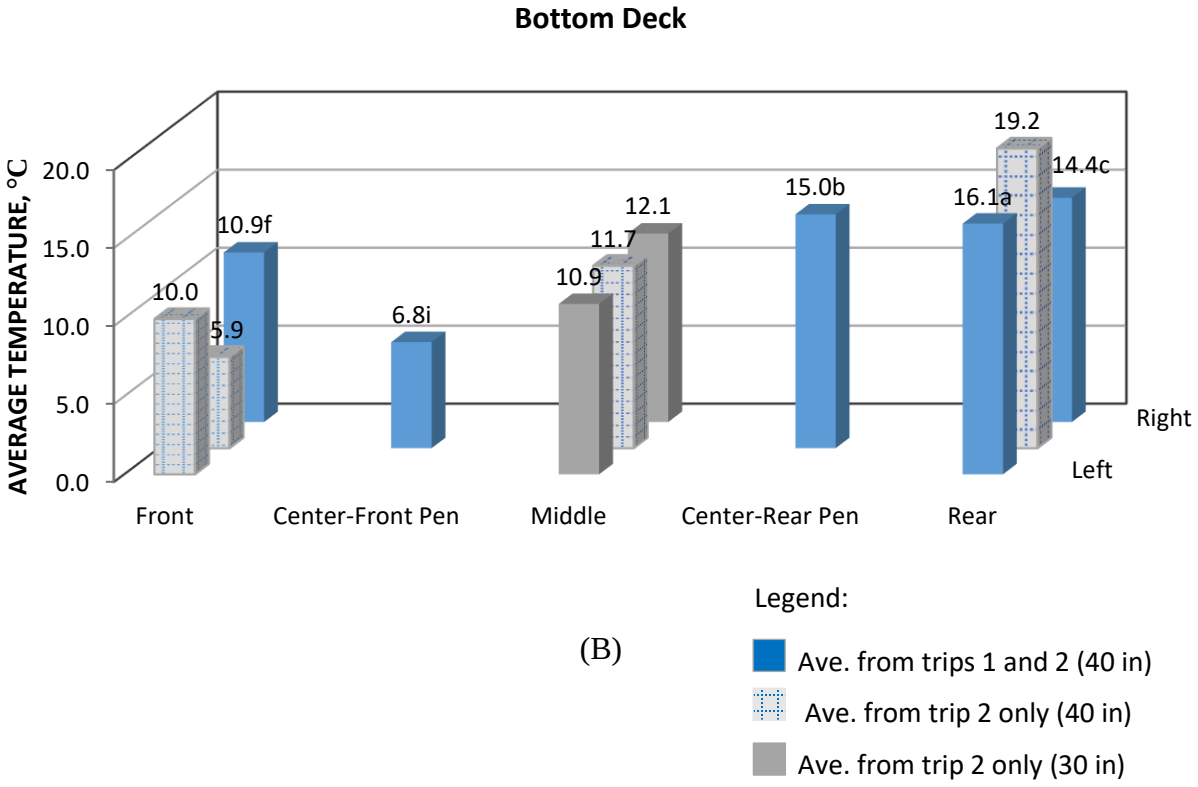
Figure 4.4 shows the distribution of average temperature inside the trailer at several locations during the stable period of the two trips. This period was defined as starting from after the trailer had travelled for approximately one hour (after loading) and determined by visual inspection of the time series shown in Figure 4.3. The stable transport period covered the longest duration in the transport process (around four hours for both trips) and was chosen to minimize the effect of travel interruptions and stationary periods in further analysis to assess the general overall trends in the resultant thermal conditions in the trailer. A series of data filtering techniques were also applied before doing analysis.

Generally, temperature inside the animal compartment of the prototype trailer increased from front to rear. Moreover, a paired t-test at 95% confidence interval of the temperature at the center of the trailer vs the average of corresponding periphery (left and right side of the trailer) temperatures showed significantly higher ($p<0.05$) temperature for the central part of the trailer. This is in agreement with the prediction done using computer simulations during the design phase of the project. Sensitivity analysis using the S2-S4 design configuration under winter conditions yielded higher temperatures at the center compared to the sides of the trailer in close proximity to the cold trailer walls (Figure 2.3B). The opposite is expected for summer conditions particularly due to higher ventilation rates during the warm weather periods where cooler inlet air from the fans can displace warm air along its path at the center (Figure 2.3A). The latter, however, was not tested using the prototype trailer.

Only the locations monitored for both the first and second trips were included in the model since the trips were considered as random variable in the general linear model analysis. Thus from Figure 4.4, not all monitoring locations for temperature were entered in the analysis for separation of means (univariate general linear model in SPSS): only seven out of 11 monitoring locations for the top deck and five out of 11 for the bottom deck were included. As shown in the figure, three of the sampling locations with significantly higher ($p<0.05$) temperatures (letter groupings a, b and c) were found at the rear portion of the bottom deck compartment: 16.1 ± 1.3°C for Location 5 – Left, 15.0 ± 1.2°C for Location 4 – Center and 14.4 ± 1.9°C for Location 5 - Right. This is in



(A)



(B)

Figure 4.4. Average air temperature during the stable transport period at different monitoring locations approximately 1 m (≈ 40 in) above the floor of the (A) top deck and (B) bottom deck of the animal compartment monitored during two monitoring trips, $n = 2$. Data labels followed by the same letter (a to i) for top deck and bottom deck combined are not significantly different

(*p<0.05*). Data labels with no letter groupings were computed from monitoring trip 2 only thus were excluded in the analysis.

comparison to corresponding locations at the top deck where temperatures were lower at 13.6 ± 1.9°C, 13.6 ± 1.2°C and 11.9 ± 1.7°C, respectively. However, it is important to note that although it was not included in the analysis, Location 5 – Middle for both decks gave highest average temperatures within respective deck compartment: 19.2 ± 1.2°C and 15.0 ± 1.5°C for the bottom and top deck, respectively. The top three lowest temperatures among all monitoring locations included in the analysis were at Location 2 – Center in the bottom deck (6.8 ± 1.2°C) and Location 1 – Left (8.0 ± 2.4°C) and Right (9.7 ± 1.8°C) at the top deck.

Although not included in the above-mentioned general linear model analysis, the mean temperature at the Location 1 - Center of the bottom deck (5.9 ± 1.4°C) was lower compared to temperature at corresponding location in the top deck (10.9 ± 1.2°C). It is important to note that averages for the said locations were based on data gathered from the second monitoring trip only where unit heater was operated at the front compartment. Thus, it is suspected that the unit heater which was installed at the top-right corner of the front compartment (refer to Figure 3.6), was able to pre-heat inlet air for the top deck only. Average bottom deck inlet temperature of 3.4 ± 1.7°C was significantly lower ($p<0.05$) than upper deck inlet temperature of 5.1 ± 2.1°C during monitoring trip 2. Table 4.4 summarizes inlet and outlet temperatures during the stable transport period of the two monitoring trips.

Table 4.4. Inlet and outlet temperatures (mean ± SD) in °C in the top and bottom decks of the prototype trailer during the stable transport period of the two monitoring trips.

Trip	Bottom Deck		Top Deck	
	Inlet	Outlet	Inlet	Outlet
1	0.0 ± 0.9	10.9 ± 1.0	-0.3 ± 0.9	12.6 ± 1.6
2	3.4 ± 1.7	12.6 ± 0.9	5.1 ± 2.1	12.4 ± 1.9

Also important to note was that the variability in temperatures across different locations inside the animal compartment at a specific time reached as high as 12C° (5.0 ± 3.0C°, mean ± SD) during the first trip and was maximum at 9.0C° (4.3 ± 2.2C°, mean ± SD) during the second trip. These high temporal differences in temperature were observed between the bottom deck front compartment and rear compartment of both bottom and top decks. This is a direct consequence of the general front to rear air flow pattern inside the trailer as dictated by the ventilation system

configuration, i.e., positive ventilation fans located at the front end of the trailer while exhaust openings are found at the rear. This is in contrast to the reported air flow pattern in conventional livestock transport trailer where differences in external pressure fields while the vehicle is in forward motion causes rear to front air movement inside the trailer (Kettlewell, et al., 2001; Ellis et al., 2008; Brown et al., 2013). This led to generally rear to front increase in temperature and other environmental parameters (e.g. moisture and CO_2 levels) in conventional trailers. However, the study of Xiong (2013) that characterized thermal environment during 43 farm-to-abattoir transport of pigs across different weather conditions showed a different air flow pattern which they found to be from the middle portion of the trailer to either front or rear of the conventional transport vehicle.

4.2.3.3 *Thermal comfort classification*

Thermal comfort classification was adapted from Xiong et al. (2015) with some modifications as it applies in the range of temperatures observed during the two monitoring trips. Thermal comfort in this study was categorized into four temperature ranges: cold (-15°C < T > 0°C), cool 0°C < T > 10°C), cool but acceptable (10°C < T > 18°C) and thermoneutral (18°C < T > 25°C).

As shown in Figure 4.5, the cool (acceptable) temperature range has the highest occurrence in most of the trailer locations. However, locations close to the fans experienced prolonged periods under the cool temperature category. For instance, front location (Location 1 – Centre) at the bottom deck during the second monitoring trip had 91.9% (approximately 6.3 hours out of the total 6.8 hours from end of loading to start of unloading during the second trip) of the time at temperatures within the 0 to 10°C range. This was expected as the location is directly facing the fan. On the contrary, locations at the rear end of the trailer for both top and bottom decks were at acceptable temperature levels for the bulk of the trips' duration. Overall, for the weather condition at which the two monitoring trips were conducted, temperatures at the front trailer locations were generally within lower temperature ranges while rear trailer locations were maintained at higher (acceptable or favorable) temperatures. It is important to characterize trailer condition this way because extended periods under cold to extreme cold conditions during winter can deplete energy reserves of the animals and compromise their overall welfare and meat quality (dalla Costa et al., 2007).

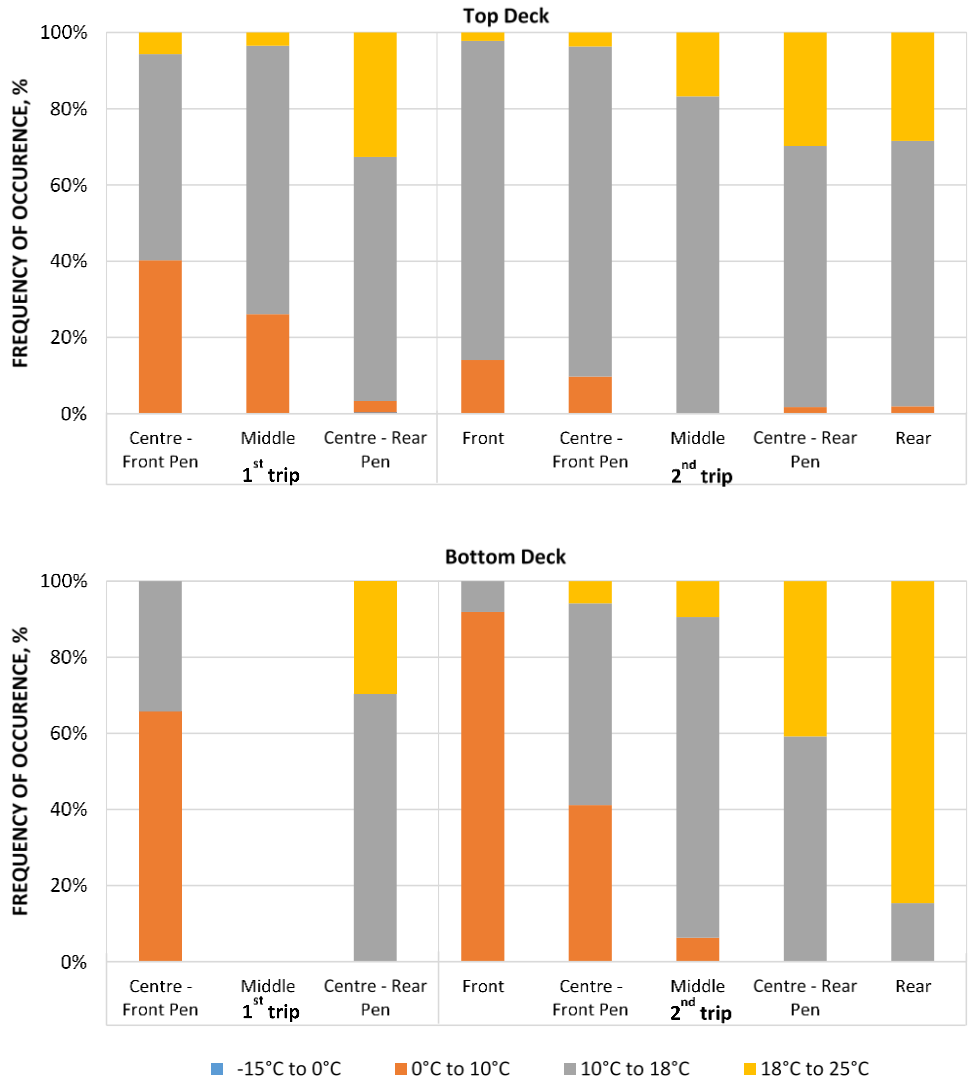


Figure 4.5. Assessment of overall trailer thermal condition by classification of observed temperatures at monitoring locations along the center of the trailer into thermal comfort categories. The color bars represent the percentage of time within each trip that the thermal condition was experienced inside the pig-laden trailer. Only temperatures starting from the end of loading at the farm and up to start of unloading at the abattoir were included in the analysis.

4.2.4 Moisture levels and distribution

To provide a more absolute measure of the moisture level inside the trailer during transport, relative humidity (%) measured were converted into humidity ratio (g/kg of dry air) (Albright, 1990). Moisture condition as depicted in the time series shown in Figure 4.6, showed generally high levels within the first to 1.5 hours from the start of loading. Interior moisture levels eventually decreased and stabilized during the main transport periods of both trips. Average humidity ratio inside the trailer during the stable transport period of the December 1 trip were 3.8 ± 0.9 g/kg of

dry air and 4.9 ± 1.1 g/kg of dry air for the bottom and top decks, respectively. Corresponding values for the December 14 trip were 4.5 ± 1.2 g/kg of dry air and 6.0 ± 1.3 g/kg of dry air, respectively. Increase in moisture level was observed upon entering the urban center where the abattoir was located. However, unlike temperature that was maintained at a higher level during the waiting period at the abattoir, humidity ratio inside the trailer dropped during the waiting period in the plant. This is attributed to expected increase in ventilation flow rate with increase in temperature upon entering the urban center and during the waiting period. This ramping up in ventilation was able to exhaust moisture generated by the animals during the travel interruption (slowing down) and eventually stationary (wait at the plant) periods. A slight increase then

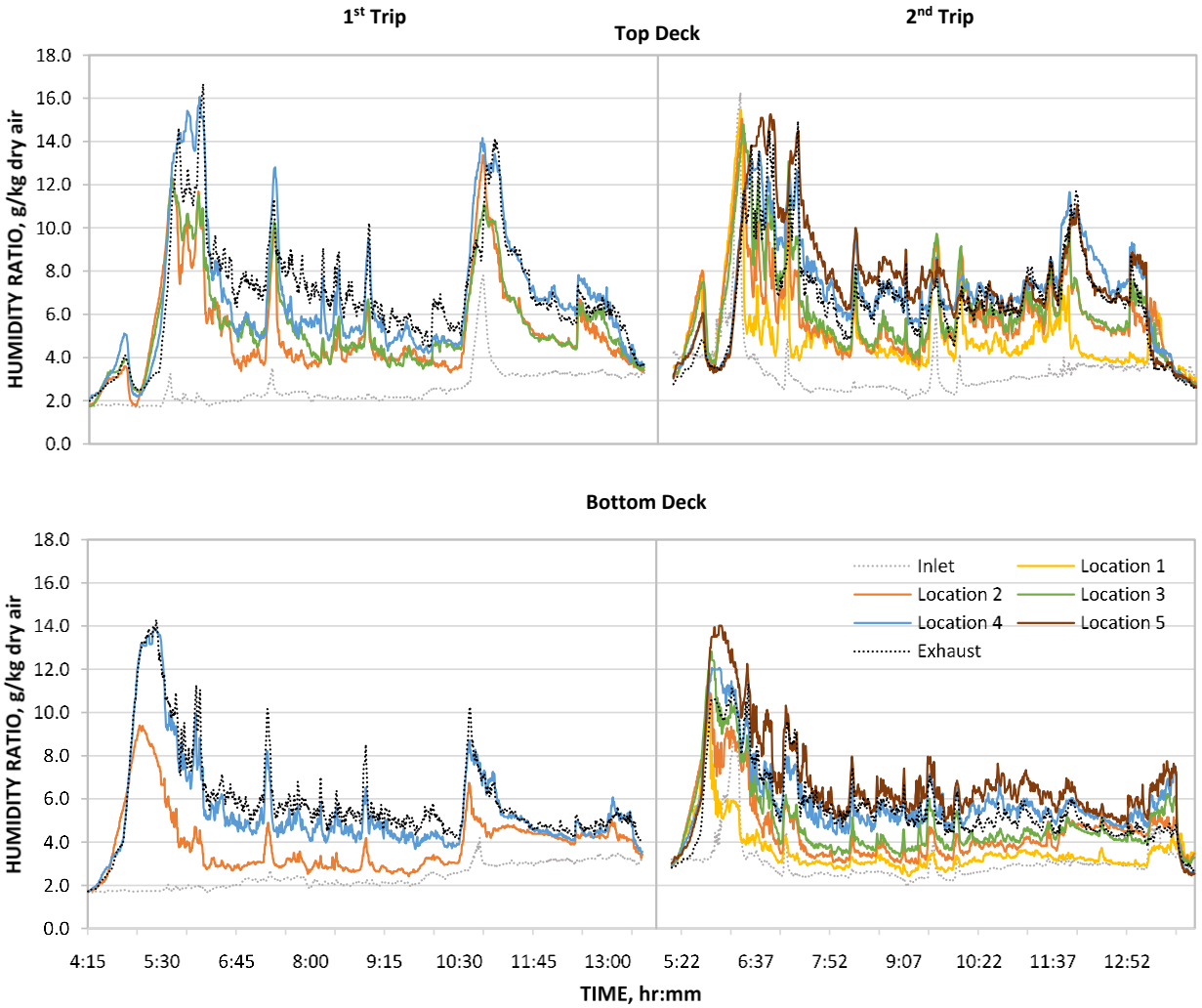


Figure 4.6. Variation in inlet, exhaust and internal trailer humidity ratio measured at different locations approximately 1 m (≈ 40 in) above the floor along the center line of the trailer top and bottom decks during the two monitoring trips. The time series represent humidity ratio levels from start of loading in the farm up to end of unloading in the plant.

followed while preparing for unloading. It is also evident that by looking at the individual time series for every trailer location, humidity ratios were increasing from front location (Location 1) to rear trailer location (Location 5). This was based on readings from data loggers for monitoring RH and temperature distributed along the center line of the trailer (Figure 3.6).

Figure 4.7 shows the average humidity ratios along five monitoring locations for both top and bottom decks of the trailer during the stable transport period. Moisture level increased from front to rear for both decks which further attest to the general front to rear air movement inside each trailer deck compartment. Considering only trailer locations included in the general linear model, Location 4 at the top deck showed significantly the highest ($p<0.05$) humidity ratio at 6.3 ± 0.9 g/kg of dry air. This, however does not discount that based on both Figure 4.6 and Figure 4.7, Location 5 had generally higher moisture level over other monitoring locations. Moreover, in contrast to temperature distribution, moisture level inside the trailer was significantly higher ($p<0.05$) at the top deck compared to the bottom. This can be explained by the lower ventilation rates maintained at the top deck (at 10% of fan capacity during the stable transport period) compared to the bottom deck for the entire duration of the study. Although the ventilation control system has relative humidity and CO_2 compensation feature as stated previously, this feature is only activated when detected temperature by the control system is equal or higher than the 16.5°C set-point.

Humidity ratio levels at the Middle to Rear locations (Locations 3 to 5) in the current study were comparatively higher than the mean humidity ratio observed during pig transport in winter using the conventional pig transport trailer in the study of Brown et al. (2011). Mean humidity ratios ranged from 1.8 to 4.9 g/kg of air across all trailer locations they monitored. Although indicative of moisture accumulation as air stream moves from front to rear, the higher moisture levels at the rear locations is not deemed detrimental during winter condition in this current trailer design as temperature in these locations are generally higher. Meaning, possibility of frostbite is minimized with high temperature – high humidity level combination at the rear trailer locations (McGlone et al., 2014b). Nevertheless, it remains important that sufficient fresh inlet air must be provided during winter periods to maintain acceptable moisture level across all locations in the trailer. The consistent increase in outlet humidity ratio compared to inlet moisture levels proves that exhaust air stream carried moisture with it (Figure 4.6).

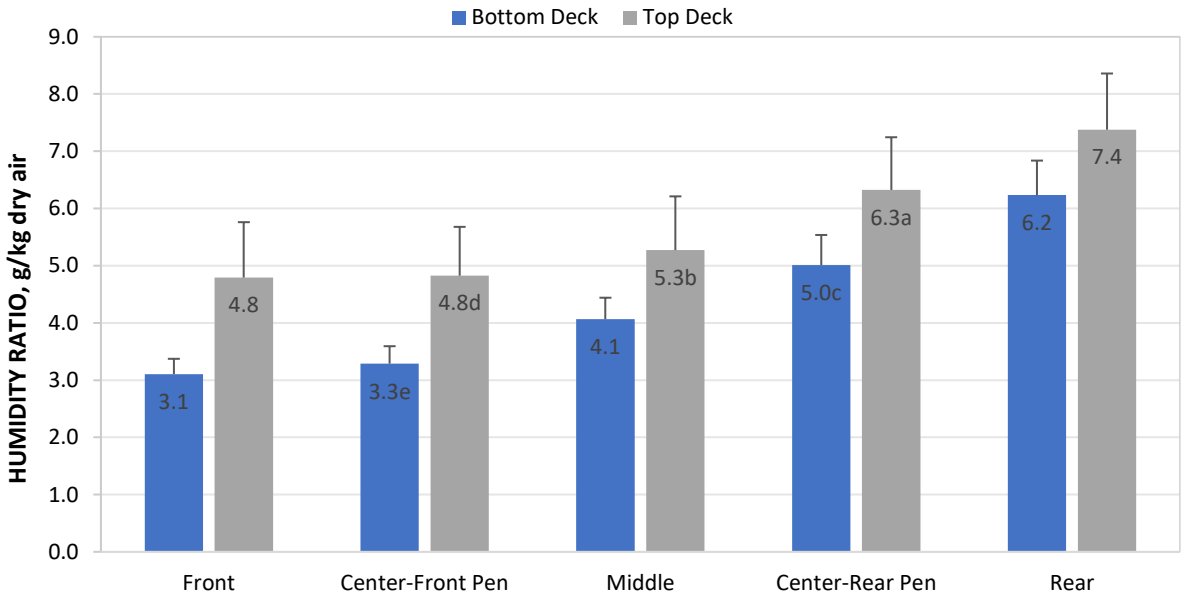


Figure 4.7. Average humidity ratio during the stable transport period measured in different locations approximately 1 m (≈ 40 in) above the floor along the center of the trailer top and bottom decks during the two monitoring trips, $n = 2$. Data labels followed by the same letter (a to e) for top deck and bottom deck combined are not significantly different ($p<0.05$). Data labels with no letter grouping were computed from monitoring trip 2 only, thus, were excluded in the analysis.

Appendix Table A.1 summarizes the temperature and humidity ratio on all monitoring locations during the two trips.

4.2.5 Carbon dioxide levels and distribution

Carbon dioxide (CO_2) level was used as an indicator of overall air quality inside the trailer during the two monitoring trips. CO_2 concentration (Figure 4.8) inside the pig-filled trailer followed the general trend exhibited by temperature and humidity ratio throughout the monitoring trips. Generally, higher physical activities such as when pigs are loaded as well as during slowdowns and other transit interruptions including traffic stops and longer stationary periods that tend to agitate the pigs, result to increase and peaks in temperature, moisture level and CO_2 levels. Equilibration during transit usually follows a few hours after loading and minutes after resuming travel, following short interruptions.

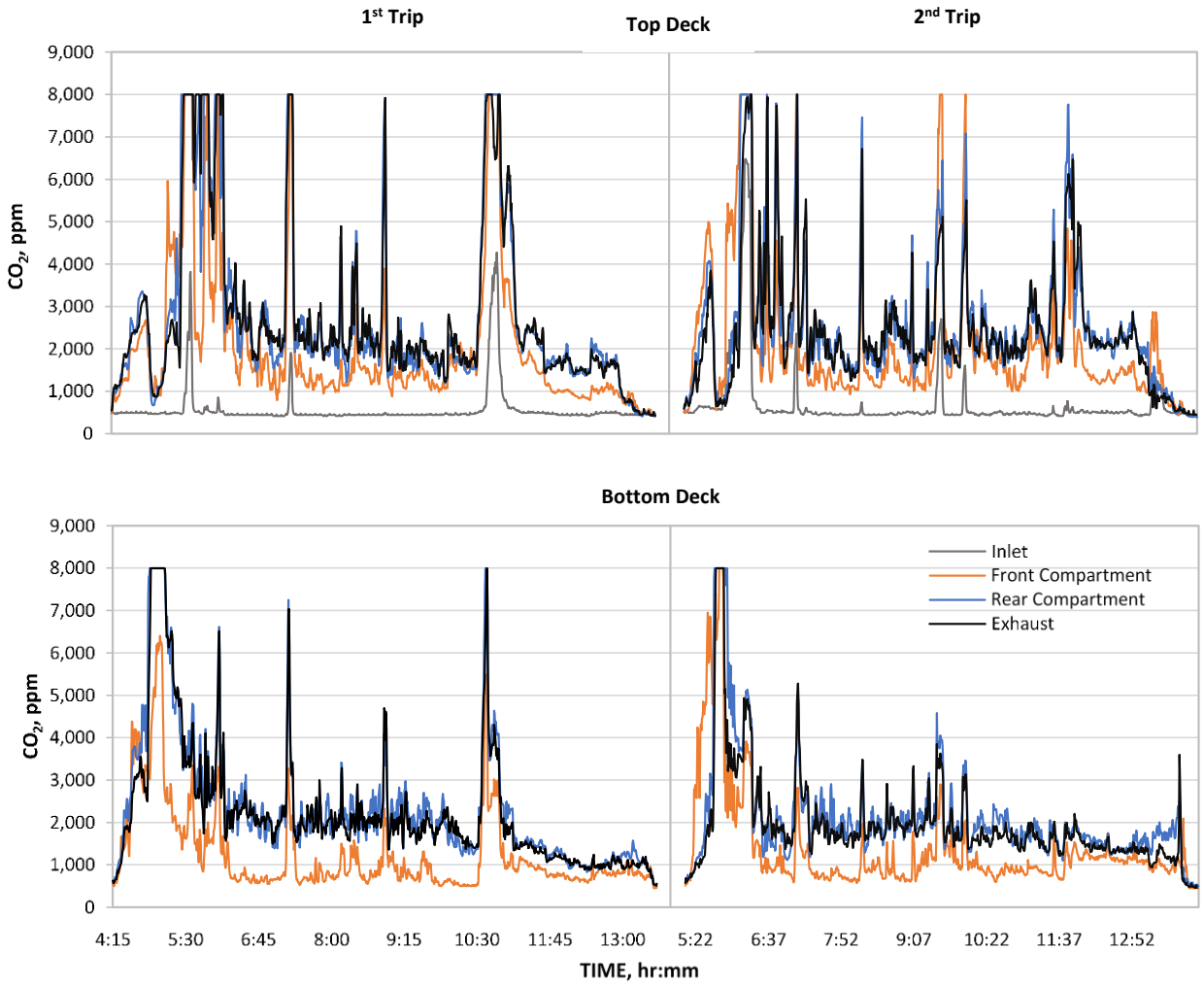


Figure 4.8. Variation in inlet, exhaust and interior CO_2 levels for both the trailer top and bottom decks during the two monitoring trips. The time series represent CO_2 levels from start of loading in the farm to end of unloading in the plant.

During the stable period of the trips, average CO_2 concentrations were 850 ± 277 and 1,999 ± 422 ppm for the front and rear pens of the bottom deck, respectively. Corresponding values for the top deck were 1,533 ± 427 and 2125 ± 518 ppm, respectively. Moreover, inlet CO_2 levels for the two trips were 462 ± 55 ppm while outlet CO_2 levels were 1,888 ± 322 and 2,209 ± 508 for the bottom and top decks, respectively. Figure 4.9 graphically summarizes these results. These values are comparable to CO_2 levels (≈ 1,900 - 2,300 ppm) observed inside a pig gestation room during cold weather trial when ventilation rates were lower (Predicala et al., 2017). In the study of Ellis et al. (2008), CO_2 concentration inside the trailer was used as an indication of the ventilation rate in different trailer compartments considering ventilation in existing commercial livestock vehicles is driven by external pressure fields and the pattern of boarding of side openings. Reported mean

CO_2 concentrations from their study during one of their summer trips ranged from 878 to 2,746 ppm. These values were comparable to the CO_2 levels in this current study despite the trailer ventilation system running at winter flow rates. Finally, CO_2 concentrations significantly increased ($p<0.05$) as the air stream reached the rear portion of the trailer. Moreover, the outlet CO_2 level suggests that the ventilation system under the given operating condition was able to remove stale air from inside the trailer.

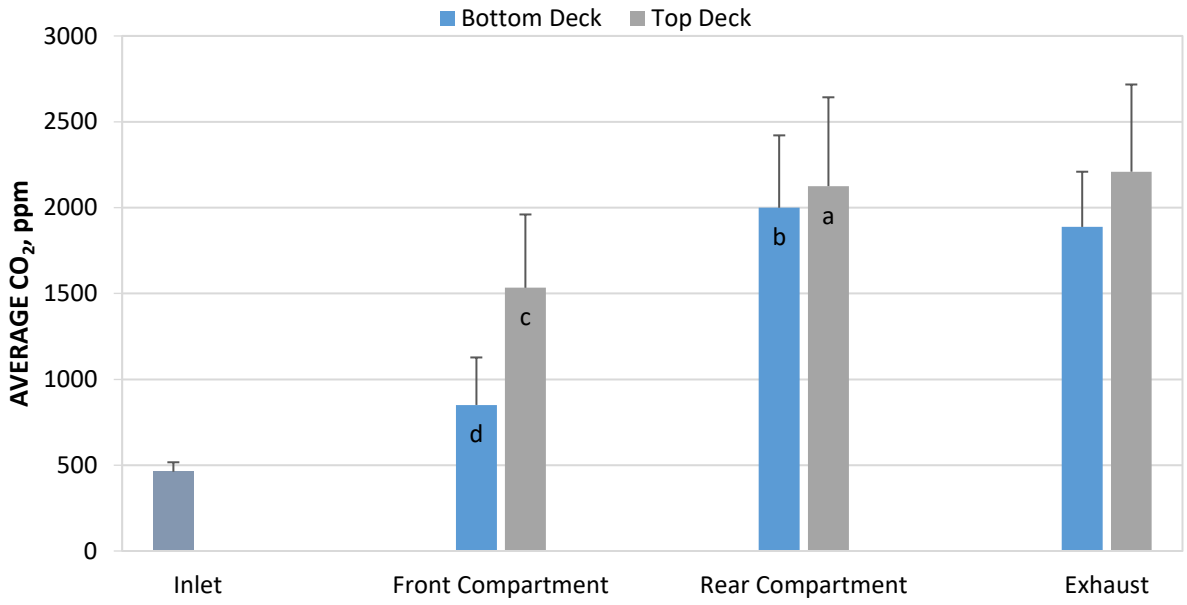


Figure 4.9. Average inlet, exhaust and interior CO_2 levels during transport (most environmentally stable period of the two monitoring trips) for both the trailer top and bottom decks during the two monitoring trips, $n = 2$. Error bars shown correspond to standard deviations of the measurements.

Monitoring of other gases, H_2S and NH_3, during the 2nd monitoring trip gave zero ppm readings throughout transport for both gases. These gases are not anticipated to be a concern in pig transport due to the limited time the animals are held in the transport container; hence, not enough time to produce alarming levels of the gases under normal transport circumstances.

4.2.6 Air speed

Air movement inside the prototype trailer was primarily driven by ventilation air provided solely by axial fans that blows air into the animal compartment at its front end. Thus, as shown in Figure 4.10, rear pen (top and bottom decks) air speeds were fairly stable throughout transit while

front pen air speeds for both top and bottom decks showed peaks in values during periods when ventilation rate increased as driven by increase in trailer temperature.

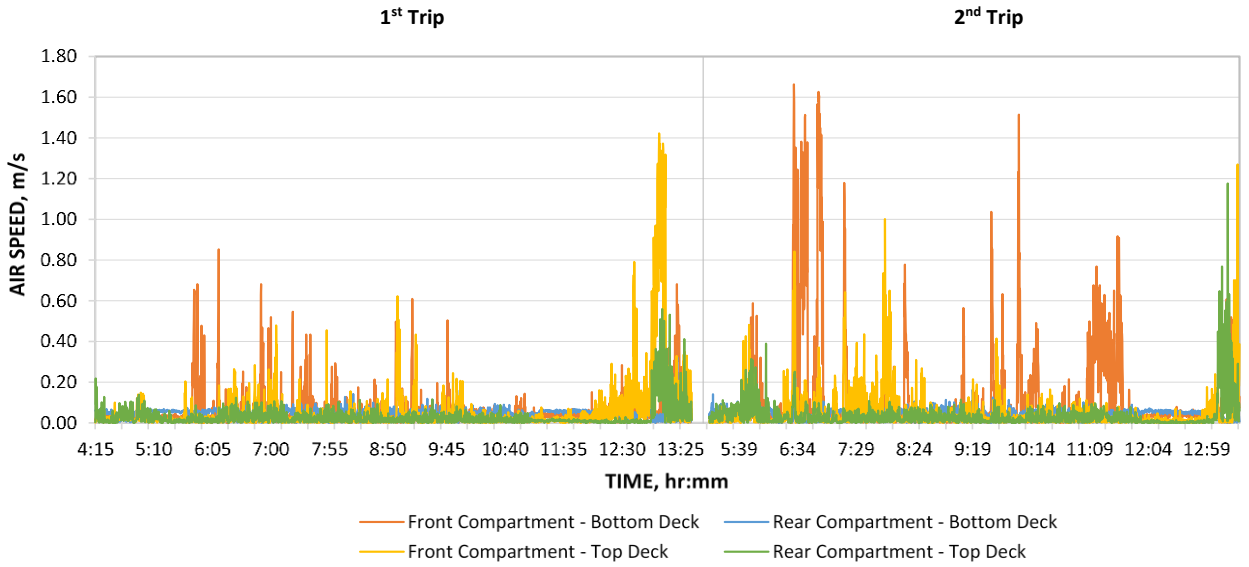


Figure 4.10. Variation in air speed inside the trailer measured at the center of the front and rear compartments of the trailer top and bottom decks during the two monitoring trips. The time series represent air velocities from start of loading at the farm to end of unloading at the plant.

Figure 4.11 gives the average air speed during the stable transport period of the two trips. As shown in the figure, the average speed at the front pens of each deck were significantly higher ($p<0.05$) compared to air speed in corresponding rear pen of each deck level. Also evident is the greater variability in air speed in the front pens compared to air speed in the rear pens. The range and mean (mean ± SD) air speeds for the four different locations in the animal compartment are as follows: 0 – 0.61 m/s (0.06 ± 0.10 m/s) and 0 – 0.11 m/s (0.04 ± 0.02 m/s) for front and rear pens of the bottom deck, respectively; and, 0 – 0.28 m/s (0.04 ± 0.05 m/s) and 0 – 0.09 m/s (0.02 ± 0.02 m/s) for front and rear pens of the top deck, respectively. For comparison, mean air speeds across monitoring locations approximately one meter above the floor during winter in a group housing gestation unit ranged from 0.03 to 0.09 m/s (Predicala et al. 2017). In addition, inlet air speeds measured by sensors located inside the housing of the ventilation fans but before the blades gave the following values: 0.07 – 5.21 m/s (2.95 ± 0.76 m/s) for the bottom deck and 0 – 4.55 m/s (1.48 ± 0.93 m/s) for the top deck. These values were computed from data sets after relevant data filtering as described in Chapter 3 was applied.

Mean air speeds at different locations inside the commercial livestock vehicles monitored in the study by Ellis et al. (2008) ranged from 0.47 to 4.10 m/s during winter. Increased air speeds during cold weather is undesirable as it can cause chill to the animals (Eigenberg et al., 2009). On the contrary, higher air speeds inside the trailer compartment during summer can help mitigate the effect of hot conditions.

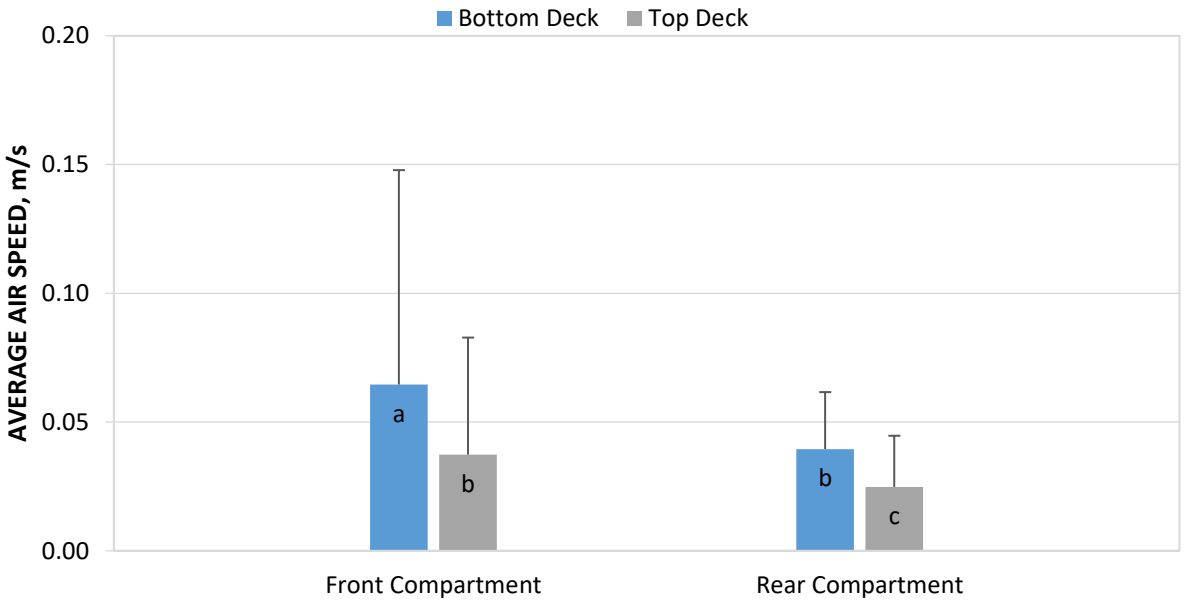


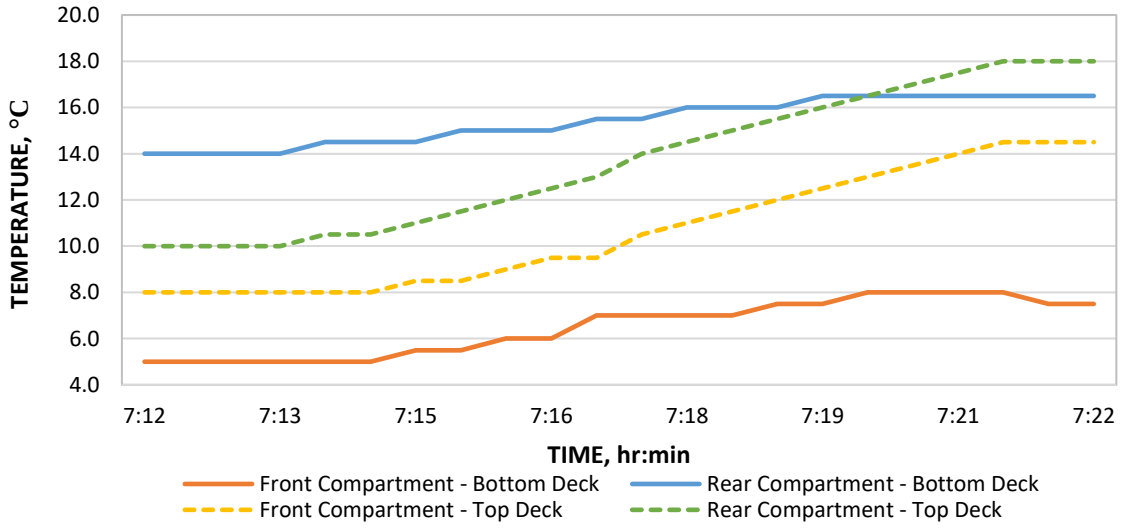
Figure 4.11. Average interior air speed during the stable transport period for both the trailer top and bottom decks during the two monitoring trips, n = 2. Error bars shown correspond to standard deviations of the measurements.

4.2.7 Effect of travel interruptions on trailer thermal condition and air quality

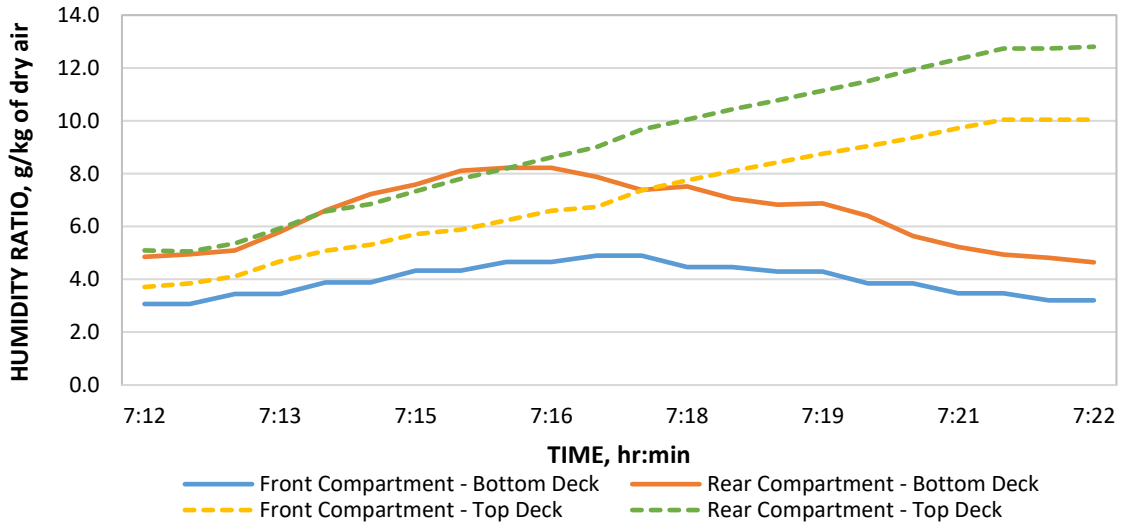
The impact of travel interruptions such as slowing down at urban centers and short breaks on the thermal condition and air quality inside the trailer with pigs loaded during the December 1 trip is summarized in Figure 4.12. The event explored through the graphs was within the stable period of the first trip when the trailer approached an intersection in a town (7:12 am) and went for a short stop afterwards (7:17 - 7:22 am).

An average temperature increase of 0.5°C/min with a 2.5 – 3°C temperature increase within a 5-minute period in the travel interruption was observed in the bottom deck compartments. A more rapid temperature rise (≈ 1°C/min) occurred at the top deck compartments with 6.5 - 8°C increase within approximately 7 minutes. While temperature gradually increased from the beginning of the travel interruption until transit resumed, change on humidity ratio and CO_2 levels,

on the other hand, peaked early in the travel interruption. At approximately around the midpoint of the 10-minute period, bottom deck humidity ratio and CO_2 levels dropped until it reached their corresponding stable levels. Top deck humidity ratio and CO_2 levels on the contrary took longer to return to their stable levels. The shorter period it took for bottom deck moisture and CO_2 levels to stabilize compared to the upper deck levels is primarily attributed to higher ventilation rates at the bottom deck because of the consistently higher temperature at the rear compartment of the bottom deck where sensors (T, RH, CO_2) used in controlling the ventilation system was installed closest to.



(A)



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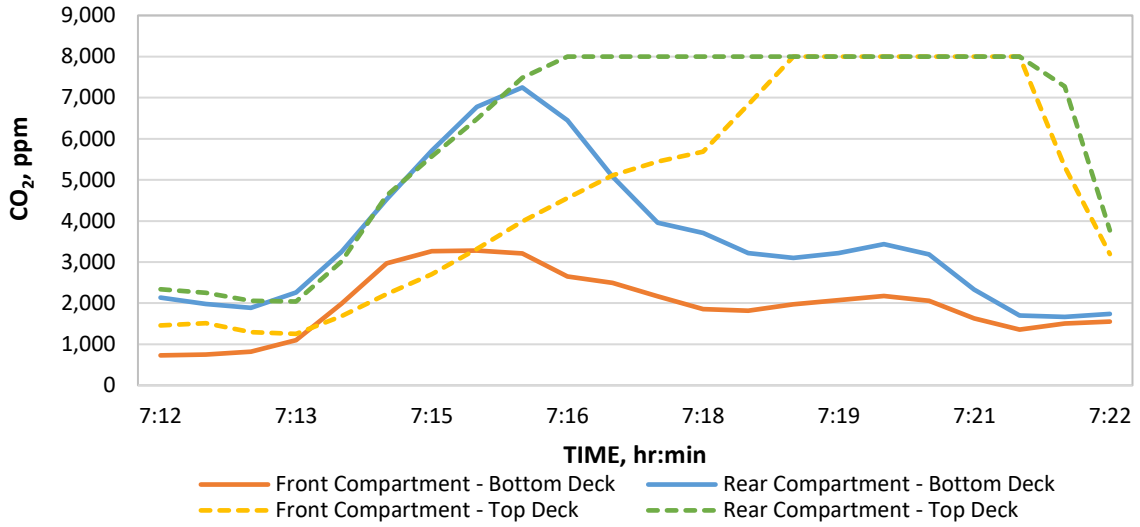

(C)

Figure 4.12. Change in (A) temperature, (B) humidity ratio and (C) CO_2 levels before and during a short stop (7:12 - 7:22 am) during the December 1, 2017 monitoring trip. Parameters were measured at the center of the front and rear pens of the trailer top and bottom decks.

Although travel interruptions showed favorable increase in temperature inside the livestock container during the mild winter condition, these however are expected to cause probable extremely high temperatures during warm summer days.

4.2.8 Ventilation effectiveness

Due to the heterogeneity of environmental conditions observed inside the livestock compartment during the two monitoring trips, location-specific HRE varied along its longitudinal plane and decreased from front to rear of the trailer deck compartments. The average HRE computed for the bottom and top decks were 1.00 ± 0.38 and 1.17 ± 0.33, respectively. Local HRE values are summarized in Table 4.5.

During heating periods when ventilation flow rates are at the minimum, the primary task of the ventilation system is to effectively remove contaminants and moisture inside an enclosed airspace such as a livestock trailer during transport. In Table 4.6, as expected, CRE values for the front pen of each deck were higher. CRE value of 4.07 for the bottom deck front pen can be explained by its proximity to the ventilation fans that supplied fresh air as well as to the relatively higher ventilation rates at the bottom deck compared to the top deck. The latter potentially displaced significant amount of CO_2 generated by the animals at the front pen of the bottom deck that led to CO_2 levels very close to that at the inlet. Slight short circuiting, i.e. CRE < 1 due to

higher concentration of CO_2 inside the livestock container than at the exhaust point, were observed at rear pens more frequently for the bottom deck, which may be attributed to non-uniform mixing of the air inside the compartment. Moreover, due to high variances in CRE values between compartments, no average CRE value for the entire deck is presented.

Table 4.5. Heat removal effectiveness (HRE) at different locations in the top and bottom decks of the animal compartment during the two monitoring trips.

Trailer Location		Bottom Deck HRE	Top Deck HRE
Front	Location 1 - Left	3.69†	2.47
	Location 1 - Center	4.95†	1.59†
	Location 1 - Right	2.58	1.71
Middle	Location 2 - Center	1.70	1.35
	Location 3 - Left	1.38†	1.41†
	Location 3 - Center	1.02†	1.12
	Location 3 - Right	1.11†	1.81†
	Location 4 - Center	0.75	0.88
Rear	Location 5 - Left	0.71	0.89
	Location 5 - Center	0.57†	0.88†
	Location 5 - Right	0.79	1.04
Average*		1.00	1.17
SD*		0.38	0.33

† Represents HRE values computed from ≈ 718 (data from monitoring trip # 2 only) time-specific HRE computations for each location while remaining mean values are averages for ≈ 1,557 time-specific HRE.
*Average HRE for the ventilation system computed from HRE values for the middle and rear portion of the trailer only. L, C and R in identifying trailer locations stand for left, center and right, respectively.

Table 4.6. Average contaminant removal effectiveness (CRE) at the center of the front and rear compartments of the top and bottom decks of the animal compartment during the two monitoring trips, $n = 2$.

Trailer Location	Bottom Deck CRE	Top Deck CRE
Front Compartment*	4.07	1.81
Rear Compartment*	0.95	1.05

* Mean CRE values for each of the four trailer compartments are averages for ≈ 1,415 CO_2 readings measured during the two monitoring trips.

No observational studies have been found so far that used HRE or CRE in assessing ventilation effectiveness in a livestock trailer due to passive ventilation applied on existing

commercial livestock trailers particularly in North America. However, HRE and CRE has been satisfactorily used as an assessment criterion in the evaluation of ventilation system designs in both human and animal buildings (van Wagenberg and Smolders, 2002; van Wagenberg, 2005; Olesen et al., 2011; Krajčík et al., 2013).

4.3 Cost analysis and design optimization

4.3.1 Cost analysis

The following cost analysis focused on the incremental costs associated with the assembly and operation of an air-filtered swine transport trailer. Table 4.7 summarizes primary cost elements in the assembly of a hypothetical 120-pig (market pigs or gilts) capacity air filtered trailer. Actual costs incurred in the construction of the 20-ft prototype air filtered trailer (60-pig capacity) were used as baseline in the estimation of cost for the full-scale air-filtered trailer. Annual operational costs, on the other hand, were estimated based on a 10-hr journey (pig transport) conducted at a maximum of two times per week.

Major incremental expenses for using an air-filtered livestock vehicle over an ordinary commercial trailer are related to purchase of generator set ($15,000), ventilation control system and fans ($11,400) and installation labor cost ($11,500). Initial cost for filters and pre-filters ($1,600) covers expense for an air filtration system with 6 sets of filter (MERV 16 filter and MERV 8 pre-filter). Space heater cost is for two units of 6-kW heater while other material cost in assembly includes hardware supplies used.

Estimated cost for purchase of a 40-ft aluminum solid wall livestock container with similar features as the prototype trailer is shown in Table 4.7. Also included in the table are costs for the addition of a hydraulic loading platform as well as having a separate compartment for the control system of the fans and housing for air filters and actual cost for the purchase of a flatbed trailer where both the livestock container and control compartment are installed. Thus, subsequent analysis including estimation of total annual costs and payback periods are based on assembly of a full and operational air-filtered swine trailer with added features such as hydraulic lift gate and hinged floors and roofs and solid aluminum walls. However, it is important to note that retrofitting of an existing commercial livestock trailer, i.e., closing and sealing of all openings and installation of air filtration and ventilation system components as required is another potential option. However, this option was not explored in this study. Total equipment and installation cost for the

hypothetical 120-pig capacity air-filtered trailer is $109,900. Moreover, operational cost per year is $9,520. The latter includes cost for diesel fuel consumption for the generator set as well as hydraulic oil for the hydraulic platform and data subscription for mobile monitoring and control of the ventilation system.

Table 4.7. Costs associated with the assembly and operation of an air filtered trailer.

Type of Expense	**Estimated Cost**
Equipment cost	
Filters and pre-filters	$1,600
Fans	$3,400
Generator set	$15,000
Ventilation system controller	$8,000
Space heater	$500
Other material costs for assembly	$3,600
Total equipment cost	***$43,600***
Total installation cost	***$11,500***
Other capital cost	
Animal container body	$43,300
Hydraulic lift gate and accessories	$11,300
Control compartment	$2,500
Trailer flatbed	$9,200
Total of other capital costs	***$66,300***
Total equipment and installation cost	**$109,900**
Operational cost	
Fuel for genset*, $/yr	$8,320
Hydraulic oil for lift gate and data, $/yr	$1,200
Total operational cost, $/yr	**$9,520**

All costs are in Canadian dollar.
*Diesel fuel cost estimated at $1.120/L. Consumption based on a 5-month heating period (i.e., space heaters are utilized) and 7-month cooling period (i.e. no heater used, but no supplemental cooling system assumed in analysis) per year.

Table 4.8 gives an estimate of the replacement cost for the filters. Assumptions were that replacement is required for the main filters every 3 years and every 6 months for the pre-filters.

This assumption was based on replacement plan applied in swine barns (Alonso et al., 2013; Reicks and Polson, 2011). A 10-year useful life (Batista et al., 2008) is considered conservative but was otherwise used in the analysis for air filters in a livestock trailer due to less moisture and dust exposure compared to those used in pig barns, as well as the downtimes (i.e., system not working) during periods when no pigs are inside the trailer. Assuming negligible cost of labor for filter replacement and maintenance (e.g. washing of filters if required), the annual cost for replacement of filters distributed over an assumed 10-year lifespan is $600.

Table 4.8. Filter replacement cost.

	Estimated Cost
Assumed lifespan, yr	10
Replacement per lifespan	3
Number of filters	6
Filter cost, $	$2,000
Total replacement cost per lifespan, $	$6,000
Total replacement cost per year, $/yr	***$600***

Simple payback period was used as final criterion in the financial analysis for this project. Also, discounted value of cash flows was neglected. Figure 4.13 shows sensitivity of the payback period for the hypothetical 120-pig capacity air-filtered trailer project as impacted by added premium (i.e., incremental price paid) ranging from $2 - $10 for every genetic stock transported using an air-filtered trailer. This assumption on premium values was based on a $1 - $10 premium estimated for every weaned pig that was PRRSV-negative in the financial impact study of air filtration in swine barns conducted by Alonso et al. (2013). Payback period computations were based on the assumption that cash inflows come solely from the premium received for every pig delivered using the air filtered trailer (with mechanical ventilation). Also, net cash inflow took into account annual operational and air filter replacement costs previously discussed. Taking for example a premium of $5/pig delivered, with two journeys served per week (personal communication) and allowing extra downtime for trailer maintenance thus transporting only 90% of the total number of weeks in a year (total of 93 journeys in a year) at 120 pigs per journey, this would translate to an annual net cash inflow of approximately $45,680 after subtracting the annual

operational and air filter replacement costs. Thus, the payback period for this premium is 2.41 years. Other modest premiums of $3/pig and $4/pig will yield payback periods of 4.70 and 3.18 years, respectively.

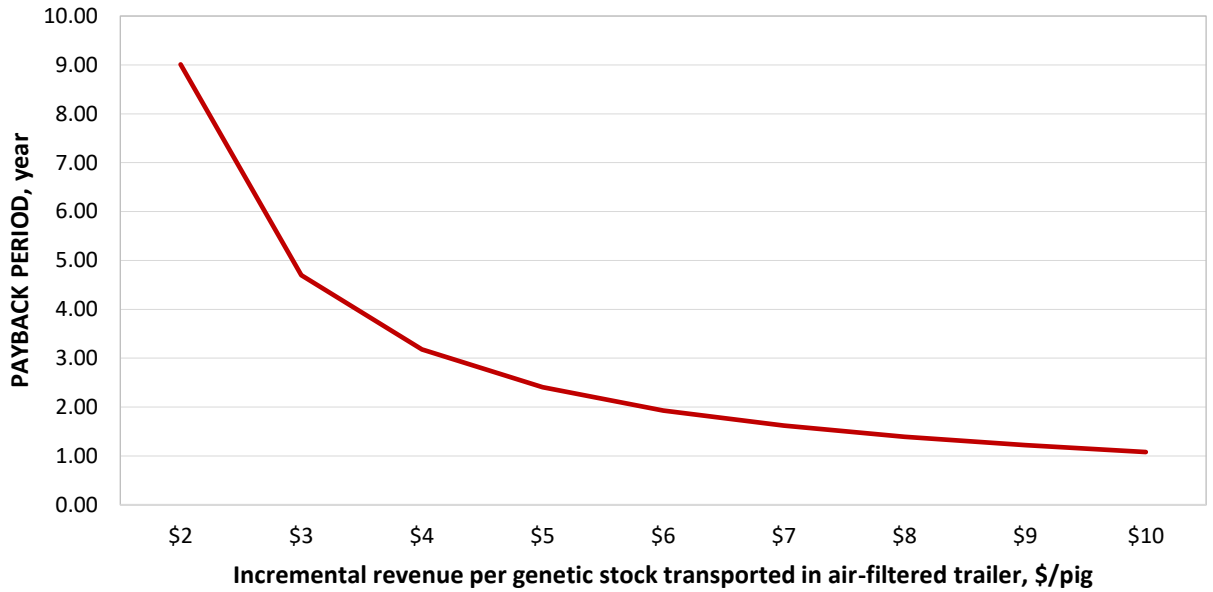

Figure 4.13. Sensitivity analysis of air-filtered trailer investment payback period considering a range of premiums (in $/yr) for every genetic stock transported.

4.3.2 Recommendations for design optimization

Based on the results and observations obtained during the evaluation of the prototype air-filtered trailer, the following recommendations for optimizing its potential in providing improved overall transport condition and biosecurity during transport were formulated.

1. Trailer body. The hinged upper deck floor and roof with air shocks for support in lifting and closing are effective features in improving working condition for herdperson during loading, unloading and other activities inside the trailer by minimizing need for bending. The latch and magnet-type mechanism in securing open or close gates were easy and efficient to use. Moreover, the relatively low deck height (≈ 3.5 ft) may have minimized opportunity for an animal to climb on top of another animal. Areas for improvement include lowering the height of the flatbed trailer platform. The current height prevented the hydraulic lift gate from reaching the ground level. Furthermore, a much tighter space may be allotted for the front compartment

(genset, fans, air filters, controller housing) compared to the 10-ft long storage container used in the prototype, thereby maximizing the livestock container space.

2. Loading platform. Safety in using the hydraulic lift gate for loading and unloading pigs can be further improved by increasing the height of its guardrails by at least one foot. This is to prevent animals from potentially jumping out of the lift gate and as added support to the herdperson when handling the animals on the platform. Also, remedies to optimize slip resistance of the lift gate floor such as modifying aluminum floor corrugations or using rubber top may be considered. However, cleanability of the platform floor should not be compromised as well.

3. Cleanability. The prototype trailer's solid aluminum walls, upper deck floor and roof that can be lifted open and minimized corners made scraping of bedding materials and trailer washing easier. Since wired sensors are located inside the animal compartment, moisture-proof housing intended for washing can be utilized. Louver type enclosure or boarding that can be quickly adjusted must be provided to close openings in the front compartment prior to washing to protect electronics and air filters inside the front compartment.

4. Ventilation system. Ventilation control systems that enable real-time monitoring of environmental condition inside the trailer such as the one used in the prototype trailer are very helpful for ensuring welfare of the animals during transport. Possibility for making quick changes in ventilation settings or bypassing pre-set ventilation control plan during travel by manually selecting desired ventilation flowrate (in % of fan capacity) for a time period should also be made possible. Although the prototype trailer was only tested at mild winter condition, it is recommended that supplemental heating be provided when outside air temperature drops below -10°C and that proper placement must be determined to ensure both upper and lower decks benefit from pre-warmed inlet air. Furthermore, use of air distribution ducts may be explored and evaluated for its technical and economic feasibility in air filtered livestock trailers. This is in response to variability in thermal condition observed between the front and rear ends of the livestock container. Additionally, full evaluation (i.e., including both warm summer and cold winter conditions) of the existing ventilation system configuration is recommended as baseline for further improvements in design.

5. Air filtration system. Although the installed air filter sets showed promising performance in the stationary test (based on overall % reduction in bacterial phage concentration), other types of filters may be considered for future evaluation.
6. Others. Incorporation of alternative air openings in case of emergency such as malfunction of generator set or ventilation controller during the course of a journey must be considered in future improved design.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions are made based on the observations obtained from evaluation of the prototype air filtered trailer:

1. The air filtration system (MERV 8 panel pre-filter and MERV 16 glass fiber V-bank filter) installed in the prototype trailer showed great potential in preventing airborne entry of pathogen to the livestock container, with the concentration of aerosolized bacteriophage inside the animal compartment reduced by 96.9% compared to initial levels upstream of the filtration system.
2. With the present air-filtered trailer design and under the weather condition it was tested, acceptable thermal condition was maintained only at selected locations inside the animal compartment. Extended low temperatures at the front locations near the ventilation fans and the overall thermal and air quality heterogeneity owing to the ventilation configuration are areas for improvement in future design optimizations.
3. Cost analysis showed financial feasibility of an air-filtered project. Substantial premiums for every biosecure pig transported with air-filtered trailer can offset cost of investment and significantly reduce payback period.

Moreover, based on observations found in this current study, the following are recommended for further considerations:

1. In order to fully characterize the potential of the developed air-filtered trailer to provide improved transport condition for pigs, it needs to be tested for colder winter and also summer conditions prevalent in Canada.
2. Improvement in overall trailer environmental condition (i.e. reducing thermal and air quality variability) may be further investigated. Using air distribution ducts or other technically feasible options for livestock vehicles with air filtration and active ventilation systems must be considered for future examinations.

3. As there still is a need to determine appropriate ventilation flowrate for livestock in transport across different weather conditions, future investigations must give weight to quantifying this parameter. More monitoring trips compared to the two done in this current study is recommended for increased confidence in observations and generalizations.
4. Although the trailer air filtration system showed high bacteriophage reduction efficiency in this study, its ultimate test must be done using actual swine pathogen aerosol with actual pigs inside the trailer for assessment of its efficacy in conditions as close to field conditions as possible.

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APPENDIX

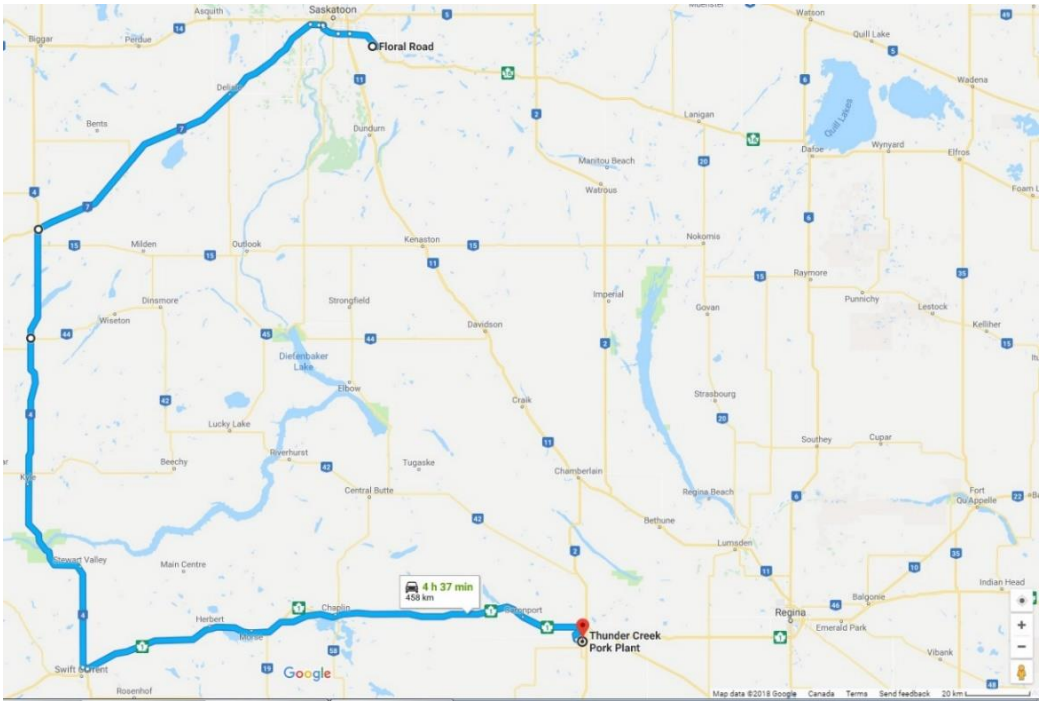

Figure A.1. Screenshot of the route taken during the two monitoring trips. Source: https://goo.gl/maps/2PLYvTw12AJ2.

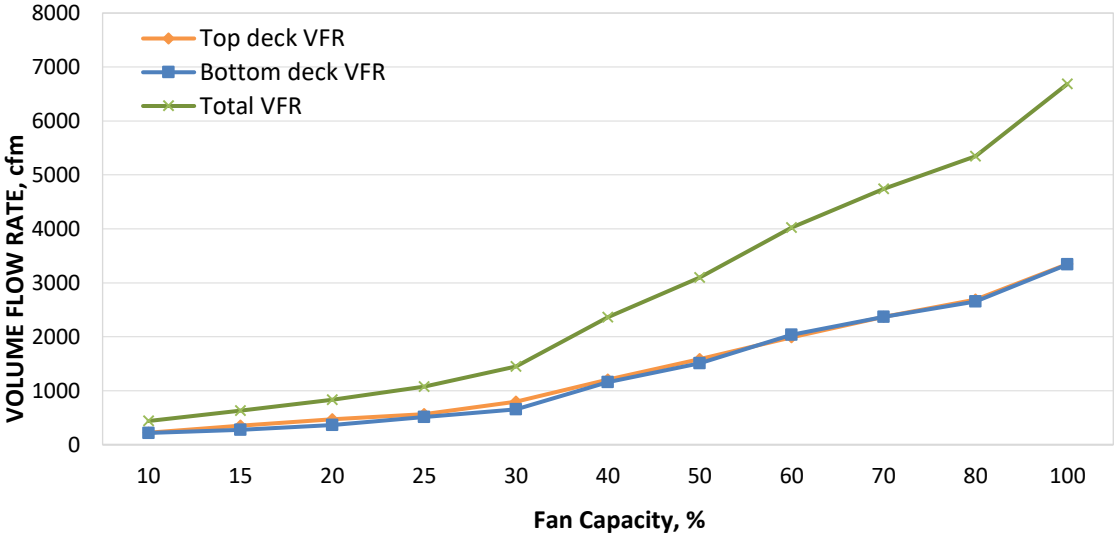

Figure A.2. Equivalent volume flow rates in cfm of the top and bottom deck fans at different % fan capacities. Figure represents ventilation flow rates after variable frequency drives (VFDs) of the ventilation system controller were adjusted to 39 Hz.

Table A.1. Mean, minimum, maximum and standard deviation of the temperature and humidity ratio at various locations inside the trailer during the two monitoring trips.

			Monitoring trip 1*				Monitoring trip 2			
			Mean	Min	Max	SD	Mean	Min	Max	SD
Temperature, °C	Top deck	Location 1 - Left	6.4	0.9	14.6	2.4	9.7	5.8	18.0	2.4
		Location 1 - Center					10.9	8.0	14.0	1.2
		Location 1 - Right	8.8	6.1	13.4	1.5	10.6	6.7	18.1	2.2
		Location 2 - Center	9.7	7.5	15.5	2.0	11.8	9.0	16.0	1.7
		Location 3 - Left					10.8	6.6	15.4	1.7
		Location 3 - Center	10.6	7.0	16.0	1.8	13.4	10.5	18.5	1.7
		Location 3 - Right					10.7	7.2	15.4	1.6
		Location 4 - Center	12.3	10.0	17.0	1.3	14.9	13.5	18.5	1.0
		Location 5 - Left	12.1	7.9	18.7	2.2	15.1	11.8	19.7	1.5
		Location 5 - Center					15.0	13.0	20.0	1.5
		Location 5 - Right	9.2	5.2	16.4	1.7	14.7	11.4	19.8	1.7
		Inlet	-0.3	-2.0	1.5	0.9	5.1	2.0	10.0	2.1
		Exhaust	12.6	8.5	17.0	1.6	12.4	8.5	18.0	1.9
	Bottom deck	Location 1 - Left					10.0	4.1	14.5	2.1
		Location 1 - Center					5.9	3.0	8.5	1.4
		Location 1 - Right	11.1	5.9	14.5	1.7	10.6	6.2	14.4	1.9
		Location 2 - Center	4.7	3.0	7.5	0.9	8.9	6.0	13.5	1.6
		Location 3 - Left					10.9	9.1	13.0	0.8
		Location 3 - Center					11.7	9.5	15.5	1.5
		Location 3 - Right					12.1	8.0	15.8	1.6
		Location 4 - Center	13.7	12.0	16.5	1.0	16.3	13.5	19.5	1.4
		Location 5 - Left	15.8	12.4	20.6	1.3	16.4	13.5	19.4	1.3
		Location 5 - Center					19.2	17.0	22.5	1.2
		Location 5 - Right	12.7	9.4	19.2	1.9	16.1	12.1	19.3	1.9
		Inlet	0.0	-2.5	2.0	0.9	3.4	0.5	7.5	1.7
		Exhaust	10.9	8.5	14.5	1.0	12.6	10.5	15.0	0.9
	Top deck	Location 1 - Center					4.8	3.4	9.6	1.0

Humidity ratio, g/kg dry air		Location 2 - Center	4.3	3.3	7.4	0.7	5.4	3.7	8.7	1.0
		Location 3 - Center	4.8	3.5	8.3	0.9	8.7	3.9	9.0	1.0
		Location 4 - Center	5.7	4.2	10.3	1.1	7.0	5.4	9.3	0.7
		Location 5 - Center					7.4	5.8	10.6	1.0
		Inlet	2.2	1.8	2.8	0.2	2.7	2.1	4.0	0.3
		Exhaust	6.9	4.5	10.7	1.3	6.5	4.5	8.7	0.8
	Bottom deck	Location 1 - Center					3.1	2.4	3.7	0.3
		Location 2 - Center	2.9	2.4	4	0.3	3.6	3.0	4.7	0.4
		Location 3 - Center					4.1	3.3	5.4	0.4
		Location 4 - Center	4.6	3.7	6.6	0.5	5.4	4.3	6.9	0.5
		Location 5 - Center					6.2	4.9	8.1	0.6
		Inlet	2.2	1.7	2.7	0.2	2.6	2.0	3.4	0.3
		Exhaust	5.4	4.1	8.1	0.6	5.3	4.3	6.9	0.5

*Monitoring locations inside the trailer that are left blank did not have data loggers assigned during the December 1, 2017 trip.