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Risk Assessment for the Transmission of Foot-and-Mouth Disease via the Transport of Raw Milk Into, Within, and Outside of a Control Area during an Outbreak

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A Collaboration between the University of Minnesota's Center for Animal Health and Food Safety and USDA:APHIS:VS:CEAH

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1. Abbreviations and Definitions

APHIS	Animal and Plant Inspection Service
CFR	Code of Federal Regulations
CIP	Clean in Place
DEFRA	Department of Environment, Food, and Rural Affairs (United Kingdom)
EMRS	Emergency Management Response System
EPA	U.S. Environmental Protection Agency
FAD	Foreign Animal Disease
FADDL	Foreign Animal Disease Diagnostic Laboratory
FDA	U.S. Food and Drug Administration
FMD	Foot-and-Mouth Disease
FMDv	Foot-and-Mouth Disease Virus
HTST	High-Temperature Short-Time Pasteurization
ICS	Incident Command System
IMS	Interstate Milk Shippers
NASS	National Agricultural Statistics Service
NCIMS	National Conference on Interstate Milk Shipments
OIE	World Organization for Animal Health, Office International des Epizooties
PI	Probability Interval
PMO	Pasteurized Milk Ordinance
RH	Relative Humidity
SLIR	Susceptible Latent Infectious Recovered Disease Spread Model
USDA	United States Department of Agriculture
VIAA	Virus Infected Associated Antigen
VS	Veterinary Services, USDA-APHIS

Acid Rinse	Part of the equipment cleaning process for stainless steel and rubber parts; removes fat, protein, and minerals and reduces bacteria.
Acute	When used in reference to a disease, describes a quick onset of symptoms, usually defined as within the last 24 hours.
Aerobiology	The study of microbiological particles including bacteria, fungi, viruses, and biotoxins that have either naturally or purposefully been introduced into the air.
Alleyway	A walking area for cattle from a barn to a milking parlor or between pens.

Bioaerosols Viable and nonviable biological particles, such as bacteria, virus, fungal spores, and pollen grains and their fragments and by-products (e.g., endotoxins and mycotoxins) that are suspended in the air. The generation of bioaerosols from water sources occurs during bubble bursting or splash, and wave action.

Biosecurity A broad range of management practices used on a dairy farm to prevent transmission of pathogens from other sources by feed, cattle, people, or other animals.

Bulk Tank A refrigerated, stainless steel vessel in which milk is cooled to 2 to 4 °C (35 to 39 °F) and stored until collected by a bulk-tank milk-tanker for shipping to the milk plant.

Carrier Animal
A convalescent or sub-clinically infected animal in which FMDv persists for more than 28 days after infection.

Clean-in-Place
A procedure that allows for the cleaning and sanitizing of equipment without dismantling—generally by means of an automated system. The U.S. FDA Grade A Pasteurized Milk Ordinance (PMO) requires CIP of milk tankers once every 24-hour period when in use.

Dairy Processing Plant
The facility that receives, stores, processes, distributes, and sells products made from milk.

Direct Load The practice of pumping milk already cooled to less than 40°F, from the milking parlor into a movable bulk-milk tanker parked at the dairy premises without the use of a farm storage tank (stationary bulk tank, milk silos). The milk tanker hauler/driver picks up this milk tanker when it is ready to take directly to processing and leaves an empty milk tanker on-farm to collect the next load of milk.

Fomite Inanimate objects such as boots, clothing, etc., that–when contaminated with a viable disease agent–can serve as a source of infection for a susceptible host.

Headspace The volume above the liquid in a closed container, such as a milk tanker.

Incubation Period
The time interval between exposure to an infective dose and the development of clinical signs. The OIE standard for FMD is 14 days.

Index Premises
First premises known to have a case of FMD during the outbreak under investigation. The true index premises contains the first true case in an outbreak and it is often not determined.

Infected Premises
Premises where a presumptive or confirmed FMD positive case exists based on laboratory results, compatible clinical signs, case definition, and international standards.

Iteration Repetition of a process within a computer program.

Manure Slurry
Mixture of organic material that is assumed to consist primarily of manure, but could also contain (FMDv from) other animal excretions such as urine or saliva

Milk Hauler/Sampler
A bulk milk hauler/sampler is any person who collects official samples and may transport raw milk from a farm and/or raw milk products to or from a milk plant, receiving station or transfer station and has in their possession a permit from any State to sample such products.

Milk House The area where the bulk-milk tank, cleaning units, and equipment are located. An enclosed facility, separate from the milking barn or parlor, in which milk is cooled or stored and in which equipment and utensils are cleaned, sanitized, and stored.

Milking Parlor
The room in or attached to a barn on a modern dairy farm maintained exclusively for the mechanical milking of cows.

Milk Plant A milk plant is any place, premises; or establishment where milk or milk products are collected, handled, processed, stored, pasteurized, ultra-pasteurized aseptically processed, condensed, dried, packaged, or prepared for distribution

Milk-Tank Truck
A milk-tank truck is the term used to describe both a bulk-milk pickup tanker and a milk-transport tank.

Open Air Factor
An atmospheric environmental factor formed by an ozone-olefin reaction that inactivates bacteria, viruses, and phages–particularly those contained in small particles (≤1 μm diameter).

Pasteurized Milk Ordinance
U.S. Department of Health and Human Services, Public Health Service, and FDA ordinance to regulate the production, transportation, processing, handling, sampling examination, labeling, and sale of Grade A milk and milk products. It also includes the inspection of dairy farms, milk plants, receiving stations, transfer stations, milk-tank truck cleaning facilities, milk-tank trucks and bulk-milk hauler/samplers.

PFU Plaque-forming unit–used in virology studies to measure the quantity of virus particles present in a sample based on the number of plaques formed per-unit-volume. In a cell culture system, the cytopathic effect (CPE) can be used to quantify infectious virus particles by the plaque-forming assay.

Premises A geographically and epidemiologically defined location–including a ranch, farm, stable, or other establishment.

Receiving Station
Any place, premises, or establishment where raw milk is received, collected, handled, stored, or cooled and prepared for further shipment.

Runovent Plastic corkscrew shaped air filter that pierces the inner lid of the hatch system on the top of a tanker. It allows breathing (air equalization) and baffles against spillage during road transport.

Sanitization Application of any effective method or substance to properly cleaned surfaces for the destruction of pathogens, and other microorganisms, as far as is practicable.

$TCID_{50}$ Tissue infective dose 50; the amount of a pathogen needed to produce pathological change in 50 percent of cell cultures inoculated–expressed as $TCID_{50}$/ml.

Transfer Hose
Milk hose carried on a tanker truck or maintained on-farm and used to transfer milk from the bulk tank into the tanker.

Zoonotic Diseases caused by infectious agents that can be transmitted between (or are shared by) animals and humans.

2. Executive Summary

In the event of a foot-and-mouth disease (FMD) outbreak in the United States, local, State, and federal authorities will implement a foreign animal disease emergency response, including restricting movement of animals and animal products. During such an outbreak, managed movement requests—including those for raw milk movement—must be supported by risk assessments that demonstrate the risk of FMD virus (FMDv) spread associated with the movements are acceptable. Proactive risk assessments are performed prior to outbreaks to improve the timeliness of emergency response and movement permitting decisions. This risk assessment is a part of the Secure Milk Supply (SMS) plan that has been developed to support continuity of markets in the dairy industry in the event of an outbreak.

This document is a proactive assessment of the risk that the movement of raw milk from a Grade "A" dairy cattle farm to processing into, within, and outside of a control area during an FMD outbreak in the United States will result in infection of susceptible animals on other premises. This risk assessment is a joint effort between the University of Minnesota, Center for Animal Health and Food Safety, and the USDA. The baseline risk assessment considers standard practices for production of Grade "A" milk in the United States and serves as a framework for: 1) evaluation of current production practices; 2) evaluation of mitigation measures; and 3) supporting decision making during implementation of a managed movement system. At the time of writing this risk assessment, there are no specific mitigation measures that will be uniformly applied within the U.S. dairy industry during an FMD outbreak, which will allow for managed movement of animals, milk, or other products.

This assessment evaluates the pathways and risk of FMDv spread associated with: 1) movement of raw milk from dairy farms producing Grade "A" milk to processing, and 2) activities associated with the movement of raw milk from farm to processing. The pathway analysis addressed how FMDv contaminated materials (raw milk and environmental media) could contaminate the hauler, tanker, and equipment while on an infected but undetected farm, leave the farm through the transport of raw milk and result in entry and deposition of FMDv contaminated materials on a susceptible farm. Current standard industry practices were considered as well as relevant current industry regulations stipulated by the PMO and States.

An on-farm disease spread model was designed and implemented and the outputs coupled with a stochastic model to estimate the quantity of virus that may be produced in milk by dairy cattle in the early stages of an outbreak, when cattle are infected and shedding virus, but have not been detected (preclinically infected). The model indicates that over 60 percent of the herd will be infectious (preclinical and clinical) by the time disease is detected on day 5 to 6 post-infection. The virus titer in both raw milk and environmental media at the time of disease detection will pose a significant risk to cattle via inhalation routes of exposure.

2.1 Key Results

The key results of this assessment are summarized below. The estimated likelihood of:

- bioaerosols emanating from a tanker and spreading infectious virus through milk collection and transport activities is estimated as low to very low.
- hauler and truck cab contamination by spilled milk at an infected but undetected farm is moderate to high.
- external tanker, storage compartments, and transfer hose contamination by spilled milk at an infected but undetected farm is moderate to high.
- virus present in milk residues left within the tanker, that has not received CIP, will result in release of virus on an uninfected farm is negligible to low.
- that viable virus will remain in the tanker after undergoing the CIP process and result in release of virus on an uninfected farm is negligible.
- direct release of virus via milk spillage from the transfer hose on an uninfected farm is moderate to high.
- direct release of virus from spilled milk contamination of the external tanker surfaces or hauler's clothing, boots, or hands is moderate to high.
- accidental milk spillage resulting in cross-contamination of a person, vehicle, or farm from travel on common roadways or other stops is low.
- the hauler and cab being contaminated by environmental media is moderate to high.
- external tanker contamination (including storage compartments and transfer hose) by environmental media is moderate to high.
- direct release of environmental media contamination from external surfaces of the tanker and hauler's clothing, boots, and hands on an uninfected farm is moderate to high.
- cross-contamination of people, vehicles, or farms via deposition of environmental media on common roads and other surfaces from external contamination of the tanker and hauler is moderate to high.

2.2 Conclusion

The risk that the movement of raw milk from a Grade "A" dairy cattle farm to processing into, within, and outside of a control area during an FMD outbreak in the United States will result in infection of susceptible animals on other premises is moderate to high. This is based on the estimated concentrations of virus in contaminated milk and environmental media that markedly exceed the minimum infectious doses for inhalation exposure in cattle, and the pathway evaluations which indicated moderate to high likelihood for transferring infectious virus via tanker activities and milk movement

3. Background for Risk Assessment

This risk assessment is a joint effort between the Secure Milk Supply (SMS) industry-working groups, the University of Minnesota's Center for Animal Health and Food Safety, and the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) Centers for Epidemiology and Animal Health (CEAH). The assessment proactively evaluates the risk of movement of raw milk during a foot-and-mouth disease (FMD) outbreak in the United States as it relates to potential spread of virus to susceptible species. In the event of a foot-and-mouth disease virus (FMDv) outbreak in the United States, the dairy industry—and local, State, and federal authorities—will implement a foreign animal disease emergency response via the Incident Command System (ICS). This response may consist of a control and eradication strategy that uses depopulation, quarantine and movement control, vaccination measures, or a combination of approaches. In order to support continuity of business during an outbreak, managed movement of raw milk may be allowed within the Control Area from dairies not known to be infected. A "request for a movement" permit must be supported by a risk assessment (or some scientifically based logical argument) to demonstrate that the risk associated with the movement of the product in question is acceptable (National Center for Animal Health Emergency Management, 2011).

Completing this type of risk assessment in a timely manner during an outbreak can be challenging. Risk assessments may take more time to conduct than the shelf life of some of the perishable ingredients or products that need to be moved. The available storage capacity might be inadequate for holding the product while the risk assessment is being completed, which may result in disposal of product. For these reasons, risk is evaluated proactively before an outbreak occurs in order to support continuity of business planning and development of mitigations to reduce the potential for disease spread during an outbreak.

The purpose of this document is to determine the risk of spreading FMDv from an infected but undetected farm in a Control Area through the transport of raw milk to susceptible livestock on other premises. Product from a known (i.e., detected) positive farm is not considered as it is assumed that the product will be restricted from movement. During an outbreak, all transport of raw milk within the Control Area may initially be stopped. This assessment takes into consideration all existing applicable regulations, the U.S. Food and Drug Agency Pasteurized Milk Ordinance (PMO), and preventive measures that are already in place.

This document is an evolving product-specific proactive risk assessment that will be reviewed and updated as necessary—before and during an outbreak—to incorporate the latest scientific information and preventive measures. When the ICS is activated in response to an FMD outbreak, APHIS (and Incident Command Staff) will review this risk assessment with respect to the situation in order to assess industry requests for movement of product.

This risk assessment does not guarantee that movement will be permitted during an FMD outbreak. This baseline assessment evaluates the pathways and current practices that would allow transport of virus from an infected but undetected farm. A subsequent assessment will evaluate proposed mitigation measures/biosecurity performance standards. Both documents are designed to provide a framework for decision makers to evaluate the effectiveness of current practices and mitigation measures in preventing disease spread through the transport of raw milk from farms to processing. The two risk assessments will provide context for consideration of additional control measures, which would allow raw milk movement for further processing into, within, and outside of the Control Area during an FMD outbreak.

3.1 Scope

This document presents an assessment of the risks associated with the movement of raw milk into, within, and outside of a Control Area to further processing; from dairy premises producing Grade "A" milk, resulting in infection of susceptible species on another premises during an FMD outbreak in the United States.

The objectives of this risk assessment are to identify the pathways and processes that present risks for the dissemination of FMDv through milk—and environmental media contaminated with FMDv—to uninfected premises through the transport of raw milk to processing. The risks are evaluated by assessing the likelihood of each pathway for transporting FMDv from an infected but undetected premises and successfully transferring it to a susceptible premises. It is assumed that if sufficient concentrations of virus can be transported from an infected but undetected farm, and gain entry onto other premises with susceptible species, there will be a high likelihood of exposure and infection of those animals. This risk assessment is applicable for standard procedures that are currently performed in the dairy industry for the production of Grade "A" milk.

3.2 Significant Assumptions Used in the Risk Assessment

This assessment is proactive in nature and cannot address specific circumstances surrounding an outbreak in detail. Therefore, we are making assumptions to establish context and applicability. This assessment is applicable to most (but not all) of the situations that may arise during an outbreak.

The risk evaluation is based on the following assumptions:

- FMD has been confirmed in the United States
- Control Area(s) have been established around Infected Premises
- Animal and product movement restrictions are in place for infected dairy farms in the Control Area
- Active heightened surveillance of livestock is occurring on farms within the Control Zone

- Dairy premises with animals not known to be infected—based on visual inspection or not currently under investigation for FMD—will be allowed to continue to move raw milk to processing to support continuity of business needs
- Dairy farms and processing plants are following Grade "A" PMO guidelines
- Farms that are allowed to move milk during an outbreak may be infected but undetected – the highest risk situation
- Dairy practices that are illegal are not addressed
- Transport of raw milk directly to customers for human consumption is not addressed

3.3 Methodology

The assessment is divided into background and analysis sections as follows:

Background

Chapter 4—PMO, Demographics, Dairy Processes, and Milk Movement

Chapter 5—Hazard Identification

Chapter 6—FMDv Spread and Outbreak Information in the Dairy Industry

Risk Assessment

Chapter 7—Conceptual Model for Virus Transport via the Transport of Raw Milk

Chapter 8—Development and Application of a Quantitative Model

- SLIR (Susceptible, Latent, Infectious, Recovered) model: estimates number of cows in each disease stage (susceptible, latent, preclinical, and clinical) on each day post-infection and the FMDv titers in the bulk tank
- Produced estimates of the bulk milk tank FMD viral titers for different farm sizes and detection levels

Pathway Analysis Evaluation

Chapter 9—Contaminated Milk Pathway

- Assessed likelihood of FMDv-infected milk that leaves an infected but undetected farm results in contamination of the environment and people at doses sufficient to cause disease on a susceptible premises

Chapter 10—Contaminated Environmental Media Pathway

- Assessed likelihood that transport of FMDv-contaminated environmental media (manure, saliva and urine mixed with soils/mud) from an infected undetected farm

will lead to exposure of an uninfected farm at doses sufficient to cause disease in susceptible livestock

- Assessed risk that once FMDv enters an uninfected farm via the transport of raw milk, susceptible animals will be exposed and infected

Chapter 11—Conclusion

Chapter 12—Acknowledgements

A risk ranking system was developed to qualitatively rank the likelihood associated with each pathway and the overall risk of exposure of susceptible livestock (**Table 1**).

Table 1. Qualitative risk-ranking system to estimate risk in the report.

Category	Descriptor
Extremely High	The event is almost certain to occur
High	There is more than an even chance that the event will occur
Moderate	The event is unlikely but does occur
Low	It is very unlikely that the event will occur
Very low	It is highly unlikely the event will occur, but it is not negligible
Negligible	The likelihood that the event will occur is insignificant, not worth considering

In this risk analysis, the uncertainty estimate is not ranked separately from the likelihood or risk estimate for the following reasons:

1. In quantitative terms, the probability of an adverse event occurring is expressed as a fraction between 0 to 1. In the case of perfect information, the risk can be placed into one of the categories in a qualitative ranking scale (negligible, low, medium, high). However, with imperfect information and high uncertainty, the risk cannot be determined to be in a single category. In this case, the analyst may be able to ascertain that the risk is within a range of categories, e.g., negligible to low.
2. In communication with decision makers, having two scales (one for risk and one for uncertainty) creates confusion in the decision process. For example, if you have negligible risk with high uncertainty, it is difficult to make a decision on whether the risk is acceptable or not. The use of a range for the risk (negligible to low), allows the decision maker to understand what could be the higher bounds of the risk estimate.

4. Pasteurized Milk Ordinance, Milk Movement, Processes, and Demographics

4.1 Pasteurized Milk Ordinance

The U.S. Food and Drug Administration (FDA) provide regulatory oversight of all milk produced for commercial sale in the United States. All fluid milk for commercial sale is Grade "A" milk, which is produced and processed under rigid sanitary regulations in approved and inspected facilities. The FDA, in partnership with the U.S. Public Health Service (USPHS), publishes the Pasteurized Milk Ordinance (PMO). These are the guideline that all fifty states have adopted as their regulation for the production of Grade "A" milk and milk products for human consumption. PMO regulations are designed for the protection of human health and do not address veterinary health issues. FMD is not a human health threat and therefore not addressed by the PMO.

The Grade "A" milk PMO regulates:

- the production, transportation, processing, handling, sampling, examination, labeling, and sale of Grade A milk and milk products
- the inspection of dairy farms, milk plants, receiving stations, transfer stations, milk-tank truck cleaning facilities, milk-tank trucks, and bulk-milk hauler/samplers
- the issuing and revocation of permits to milk producers, bulk-milk hauler/samplers, milk-tank trucks, milk transportation companies, milk plants, receiving stations, transfer stations, milk-tank truck cleaning facilities, haulers, distributors, and the fixing of penalties

The USPHS/FDA's recommended Grade A PMO is the basic standard used in the voluntary Cooperative State-USPHS/FDA Program for the Certification of Interstate Milk Shippers; a program participated in by all fifty States, the District of Columbia, and U.S. Trust Territories. The USPHS/FDA does not have legal jurisdiction in the enforcement of milk sanitation standards, except on interstate carriers and milk and milk products shipped in interstate commerce. It serves solely in an advisory capacity and its program is designed primarily to assist State and local regulatory agencies. When the PMO is adopted locally, its enforcement becomes a function of the local or State authorities.

The Grade A PMO is recommended for legal adoption by States, counties, and municipalities, in order to encourage a greater uniformity and a higher level of excellence of milk sanitation practice in the United States. An important purpose of this recommendation is to facilitate the shipment and acceptance of milk and milk products of high sanitary quality in interstate and intrastate commerce. The Standards portion of the PMO is separated into sections that address:

- procedures for construction of dairy related facilities and equipment

- milking of cows and procedures for abnormal milk
- cleanliness of cows, dairy related personnel, and dairy related facilities
- cleaning and sanitization of utensils, equipment, and vehicles
- temperatures of milk and milk products
- sampling and transfer of milk
- protection of milk from contaminates

The University of Minnesota has developed a separate document that summarizes the major sections of the PMO.

4.2 Demographics

Information on the distribution and changes in size of dairy herds in the United States from past and current U.S. Census of Agriculture Statistics (NASS 2006, 2010) is presented in Appendix A. This section presents information on the number of dairy operations, percent inventory and milk production by farm size, the top 10 dairy producing States, and milk production and farm structure in major dairy States.

4.3 Dairy Processes and Milk Movement

Dairy farms use a variety of barn and milking parlor designs. Most common is a pipeline for milk collection in a tie stall or stanchion barn, or free-stall housing with a milking parlor. Cows are milked twice per day, with a small minority of herds milking three times per day. The milk from each animal is pumped through vacuum lines and mixed with milk from other cows in a bulk tank. Milk is cooled by plate chillers prior to storage in the refrigerated bulk tank. Raw milk for pasteurization shall be cooled to 10 ºC (50 ºF) or less within four (4) hours or less after starting milking operations and milk shall be cooled within two more hours to 7 ºC (45 ºF). The bulk-milk storage tank temperature should not exceed 7 ºC (45 ºF) after that point. Milk may also be pumped into and stored in direct-load trailers that serve as a transportable bulk-milk (storage) tank parked at the dairy premise. The milk hauler picks up this milk tanker when it is full and transports it directly to the processing plant. An empty tanker is left on the farm to collect the next load of milk.

Bulk-raw milk may be transported directly to the milk plant or it may be held at a transfer station where it is pooled with other bulk-raw milk loads. Milk is picked up from a farm daily every other day depending on storage capacity at the farm. Tankers may make multi-farm pick-ups, in which milk is collected from several farms and commingled prior to transport and unloading at the plant. At a transfer station, raw milk is unloaded from smaller milk trucks into holding silos. The commingled milk is reloaded into larger over-the-road tanker trucks for delivery to processing plants. Transfer stations are not directly addressed in this risk assessment as the risk pathways are expected to be similar to unloading of milk at a processing plant.

On the farm, the bulk-tank milk contents must be completely pumped into the tanker, as partial unloading of milk is not permitted. For tankers with truck mounted transfer hoses, the driver caps and winds up the transfer hose and places it back into the storage compartment on the tanker after milk pumping is finished. Residual milk is often present within the hose. Larger dairies generally have a transfer hose and pump that remains on-site. The milk-house floor is washed down with clean water to remove spilled milk and the bulk tank clean-in-place (CIP) unit is turned on, which includes cleaning of the on-site transfer hose. This process is conducted each time the bulk tank is emptied.

The sanitary requirements for transportation of raw milk from farm(s) to the processing plant are presented in each individual State's Department of Health regulatory documents. The duties and responsibilities of the hauler are outlined in each State's milk hauler's manual. In general, the hauler's duties include observation, measuring, sample collection, and pumping of milk—in addition to completing associated documentation. It is the responsibility of the hauler to collect a representative sample of milk from each farm's bulk tank prior to transferring milk from the bulk tank to the milk tanker. The samples are collected, kept in an insulated and chilled sample box, and delivered to the processing plant, receiving station, or transfer station. Official paperwork is transported with the milk and samples to the laboratory at the processing plant.

Upon arriving at the milk processing plant, the tanker enters a receiving area. At some plants, the tanker moves through a weigh station prior to entering the receiving area and is re-weighed after unloading. A plant technician from the quality assurance department collects the individual farm milk samples along with a representative milk sample from the tanker load for quality control tests. Prior to unloading, the temperature of the milk within the tanker is checked and the tanker raw-milk sample is analyzed for beta lactams, titratable acidity, fat, proteins, total solids, temperature, and sensory evaluation (D. Davis, personal communication, February 2012). Once the initial laboratory tests are completed, the tanker unloads the milk into storage silos. Milk tankers may unload their milk at the plant and go back out to pick up milk from additional farms prior to having the tanker cleaned internally by a CIP system. The PMO requirement for internal cleaning of the tank is once every 24 hours. The inside of the tank, vent gasket, and the transfer hose is washed using an automated system with a series of washes and rinses to clean and disinfect the tank. A representative protocol for CIP is presented in Section 9.4.

5. Hazard Identification

This section of the risk assessment addresses background information on the virus from recent publications to provide the most current knowledge and opinions about the science and epidemiology of the virus (Alexandersen et al., 2003c; Honhold, 2011; Alexandersen and Mowat, 2005; Kitching et al., 2005; Kitching et al., 2007). The virus characteristics, transmission, disease epidemiology, incubation, and clinical signs are presented below. The rest of the Hazard Identification section is presented in Appendix B and covers the following topics:

1. Agent and Host Range
2. Geographic Distribution
3. Human Disease (Zoonotic Potential)
4. Resistance to Chemical and Physical Agents
    - Background on stability of FMD in milk products
    - Laboratory studies on pH and temperature inactivation of virus in tissue suspension
    - Studies on pH and temperature inactivation of virus in milk systems
    - Thermal inactivation via pasteurization simulation studies
    - Heat inactivation
    - Chemical inactivation
5. Environmental Persistence
6. Laboratory Diagnosis

5.1 Virus Characteristics

A substantial volume of information is available on the characteristics of FMD virus and the disease it causes. FMD is a severe, clinically acute, vesicular disease of cloven-hoofed animals including domesticated ruminants, pigs, and more than 70 wildlife species (Coetzer et al., 1994). FMD virus is a member of the *Aphthovirus* genus of the family *Picornaviridae* and is a non-enveloped virus, approximately 26 nm in diameter with an icosahedral symmetry. It contains a molecule of single stranded RNA and 60 copies of each of the four structural polypeptides (VP1-VP4). There are seven FMDv serotypes (O, A, C, Asia 1, and South African Territories 1-3 (SAT 1-3)).

The serotypes are antigenically distinct; immunity to one does not offer cross protection against the other serotypes. All of the serotypes produce clinically identical disease in livestock, but their individual epidemiological behaviors are unique making it impossible to predict specific characteristics of an outbreak. Therefore, the virus should be considered as seven separate clinically indistinguishable diseases (Kitching, 1998, Kitching et al., 2005). Some strains of each

serotype appear more virulent, and some strains are shed in large quantities as respiratory aerosols from infected animals, while others are not. “It is unwise to consider FMD as a single disease which behaves in a pre-determined manner. To do so can be an economically and socially expensive mistake” (Kitching et al., 2005).

5.2 Transmission

The most common mechanisms by which FMDv is spread (Honhold, 2011, Alexandersen et al., 2003c, Alexandersen and Mowat, 2005) includes:

- Direct contact with infected animals and movement of animals between farms
- Contact with secretions from shedding animals─ exposure to secretions or mechanical transfer between groups by fomites (hands, footwear, clothing, vehicles, and equipment) and subsequent virus entry through cuts or abrasions in the skin or mucosa
- Ingestion of FMDv contaminated animal products (meat) by pigs through swill feeding
- Spread by wind—an uncommon event that requires the simultaneous occurrence of particular epidemiological and climatic conditions

Animals in the infectious phase of the disease can be expected to shed the virus for many days or weeks. Depending on the extent of livestock movements and the number of animals contacted, the disease can spread to multiple herds and animals before clinical signs are sufficiently severe to be noticed and reported. The profound delay in diagnosis of the index case of FMD, as a consequence of an owner’s failure to recognize signs of FMD, contributed immensely to the magnitude of the United Kingdom epidemic in 2001 (Scudamore and Harris, 2002). Such delays in diagnosis related to disease reporting were also seen at the onset of the epidemics in Taiwan in 1997 (Perez et al., 2004) and in Canada in 1951-1952 (Daggupaty and Sellers, 1990).

5.2.1 Direct Contact and Contact with Secretions

Close contact between animals leads to direct transmission of virus from infectious to susceptible animals through droplets or droplet nuclei secreted in respiratory secretions (Donaldson et al., 2001). The amount of virus produced in respiratory secretions is dependent on the virus strain and the host species. Cattle, because of their large respiratory volume, are primarily infected through inhalation exposures. In contrast, pigs are more resistant to inhalation exposure and are primarily infected through ingestion of contaminated food (Donaldson and Alexandersen, 2001). Ruminant animals need a much higher viral dose for infection via ingestion and are seldom infected naturally by this route (Honhold, 2011, Alexandersen and Mowat 2005). The relative importance of each mode of virus transmission is dictated by the particular characteristics of the outbreak. Under typical conditions, direct contact is the most likely route for exposure (Donaldson and Alexandersen, 2002; Gloster et al., 2008).

Respiratory excretions, and other body excretions containing the virus, can lead to exposure of susceptible animals through contamination of objects and surfaces (both porous and non-porous) (Boone and Gerba, 2007). Virus aerosolized from coughing animals, direct contact with animal secretions, or splashing of milk, urine, or feces can lead to generation of fomites. These contaminated sources may transfer virus to animate (human hands) and inanimate objects or to other fomites, leading to further spread of disease.

Clinical examinations and blood collection by veterinarians and other personnel significantly increase the risk of indirect spread of virus by equipment and personnel during an epidemic. Surgical equipment, brushes, and artificial insemination (AI) equipment have been implicated in the transfer of FMDv in previous outbreaks. The use of animal transport vehicles that have not been cleaned and disinfected, and the transport of FMDv-contaminated materials, are mechanical means for spread of the virus. The use of high-pressure hoses to clean contaminated areas may generate aerosols through splashing of infected milk and body secretions. The spraying of infected slurry on pastures is another route for generation of infective aerosols (Alexandersen et al., 2003c). Potential secondary routes for virus spread include splashing of contaminated milk or fecal slurry, rain falling onto contaminated soils, and dissemination of aerosolized virus from tankers during filling activities on the farm (Alexandersen et al., 2003c). The quantities of airborne virus which these procedures could potentially generate have not been estimated (Donaldson, 1986).

5.2.2 Ingestion

An important route for viral spread and disease outbreak is through the feeding of infectious products (e.g., raw milk and uncooked meat) to susceptible livestock, typically pigs. The 2000 South African and 2001 United Kingdom FMD outbreaks were linked to the feeding of uncooked contaminated products and fodder to pigs (Alexandersen et al., 2003a; Knowles et al., 2001; Kitching et al., 2005). Feeding of raw milk to calves and pigs has also been shown to be a route for spread of the virus.

5.2.3 Behavior of FMDv Aerosols

The airborne transmission of FMDv aerosols is complex. Windborne transport of virus can occur under specific epidemiological, climatic, and meteorological conditions, but is very uncommon. The climatic conditions which favor long-range transport of virus from animal sources are very specific. They include a high degree of atmospheric stability, relative humidity (RH) above 60 percent, low to moderate wind speeds, low precipitation, and flat topography, which results in low levels of turbulence and minimal mixing. In contrast, high wind speeds, or a convective atmosphere, will result in rapid mixing and much lower downwind concentrations (Gloster et al., 2005).

Prevailing climatic conditions, particularly wind speed and the vertical temperature structure are major determinants of physical decay of aerosols. The roughness of the surface over which air

passes influences the amount of turbulent mixing. Topographical features will determine the direction the plume travels. The survival of FMDv in plumes is likely across seaways, as the surface turbulence is low and concentrations of airborne particles can be maintained for greater distances than over land (Gloster et al., 2005).

The stability of FMDv is affected by radiation, RH, temperature, and weather factors. RH is the major meteorological determinant affecting virus survival. It has been established that the virus is stable in aerosols at a RH above 60 percent and at temperatures below 33 °C (91 °F). Sunlight and a pollution complex termed "the outside air factor" have minimal direct effect on virus survival (Donaldson and Ferris, 1975). The aerosols, once airborne, are subject to both physical and biological loss. Biologically, the virus may become inactivated if the RH of the air falls below 60 percent or the pH of the virus containing aerosol particle's water vapor becomes acidic or alkaline (Gloster and Alexandersen, 2004). In the absence of turbulence, particles greater than 10 µm are likely to be removed from the atmosphere within minutes—smaller particles may remain airborne for several hours and be carried many kilometers in the wind (Gloster et al., 2007).

In comparison with other livestock species, cattle are the largest overall producers of FMDv from all secretions/body fluids combined and are probably the main source for environmental contamination. They produce large volumes of FMDv in the epithelium of the tongue, which often sloughs off during clinical disease, as well as in saliva, urine, feces, and milk in comparison to other species. Cattle are extremely susceptible to infection by aerosol exposure to virus due to their large respiratory volume and may become infected at concentrations of FMDv as low as 0.06 $TCID_{50}$ per cubic meter of air (Donaldson et al., 2001).

Pigs are recognized as the largest producers of aerosolized virus, excreting virus concentrations in the range of $10^{5.6}$ to $10^{8.6}$ $TCID_{50}$ per pig/day (Alexandersen and Mowat, 2005). Although pigs are large aerosol producers they are very resistant to infection by this route. If a large group of pigs becomes infected with an appropriate viral serotype, the group can excrete large volumes of aerosolized virus, which can be transported to farms downwind and constitute a risk to sheep and cattle. Ruminants excrete less virus in their breath (10^4 to 10^5 $TCID_{50}$/day) compared to pigs, but are highly susceptible to infection via the inhalation route (Alexandersen and Mowat 2005).

5.3 Disease Epidemiology

Replication of FMDv takes place in the pharynx and the main amplification the subsequently takes place in the cornified epithelium of the mouth, skin, feet, and mammary glands (Alexandersen et al., 2003b,c). In the early stages of disease, infected animals shed the virus in all of their excretions and secretions; some of these fluids can contain significant titers prior to the development of clinical signs. Peak production of the virus coincides with the onset of clinical signs. Around day 4 or 5 of clinical disease, a sharp decline in viral concentration and excretion occurs due to development of a circulating antibody response.

FMDv is excreted in milk starting 1 to 2 days before clinical signs appear and through the clinical phase, in a pattern that largely mirrors the viremic profile) (Burrows, 1968). Urine and feces contain lower concentrations of virus compared to serum, nasal secretions, saliva, and milk and pharyngeal fluids. Feces contain only small amounts of virus while still present within the body, but upon defecation they are likely to be contaminated further by admixture with desquamated lesion material, vesicular lymph, saliva, milk, and urine on the ground (Parker, 1971). The high levels of virus in all secretions and excretions can lead to massive contamination of the environment. These infectious media (milk, vesicular fluid, saliva, urine, and feces) are important as sources of contamination for clothing, boots, and exposed skin and can be transferred via handling of susceptible livestock.

Calves that drink FMDv infected milk may be exposed to the virus through inhalation of milk droplets as well as by ingestion (Kitching, 2002; Ryan et al., 2008). The classic vesicular signs of FMD are occasionally noted to a milder degree in young animals. Young calves may die before the appearance of vesicles because of the predilection of the virus to invade and destroy cells of the developing heart muscle (USAHA 2007; Kitching 2002). Calves are more likely to be sub-clinically infected and are less likely to be identified as infectious sources, therefore potentiating the spread of virus (Alexandersen et al., 2003c; Orsel et al., 2009). The morbidity of FMD is nearly 100 percent in susceptible animals of all age groups. However, mortality in younger animals ($\geq$ 20 percent) is higher than that seen in adults (1 to 5 percent).

Non-vaccinated cattle are very susceptible to FMD. Viral spread among cattle and among pigs is usually rapid, so that frequently 90 percent of the animals in a herd may be showing clinical signs (Kitching et al., 2007). Approximately 50 percent of cattle can become carriers of the virus after infection, regardless of their vaccination history. A carrier is defined as an animal from which FMDv can be recovered after 28 days. The establishment of the carrier phase is dependent on the serotype and strain, while the duration of infection is dependent on the ruminant species affected and the individual. Anecdotal and field evidence indicates that carrier cattle can cause new outbreaks of disease, but this situation has not been proven under controlled conditions. The potential for these carrier animals to cause additional outbreaks of FMD is extremely controversial and not proven (Kitching, 2002).

The estimated minimum infectious doses, which have been reported to produce clinical disease in cattle, sheep, and pigs, are presented in **Table 2**. This table has been cited extensively in the literature and is based on experimental studies that were designed to reliably infect animals for research purposes. These studies were not specifically designed to determine minimum infectious doses of virus. The values cited are not absolute values, but are estimates based on different experimental designs in which small groups of animals and different methods of virus introduction were used; therefore, they are not necessarily directly comparable (Alexandersen et al., 2003c) and are not directly applicable for risk assessment work.

Ruminants are highly susceptible to airborne exposure and may be infected experimentally by airborne exposure to as few as 10 $TCID_{50}$. Ruminants are relatively insensitive to experimental infection by oral exposure to virus (Donaldson et al., 1987), requiring doses of 10^5 to 10^6 $TCID_{50}$ (Sellers, 1971). Animals with abrasions of the epithelium in and around the mouth may be infected by smaller doses (Donaldson, 1987). Nasal instillation has been used in experimental studies to initiate infection. Generally the minimum infectious dose via nasal instillation is much higher than the aerosol dose because only a small proportion of the nasal instillation dose will reach the pharynx; most of it will be swallowed or exit via the nares (Alexandersen et al., 2003c; Sellers, 1971).

Table 2. Selected estimated minimum infectious doses for cattle, sheep, and pigs by route of exposure (Alexandersen et al., 2003c, Donaldson et al., 1987).

	Minimum Infectious Doses*		
Species	**Inhalation**	**Nasal Instillation**	**Oral**
Cattle	10	10^4-10^5	10^5-10^6
Sheep	10	10^4-10^5	10^5-10^6
Pigs	>800	Unknown	10^4-10^5

*Doses are given as $TCID_{50}$ (50 percent bovine thyroid tissue culture dose endpoint estimates).

Sellers and Gloster (2007) reviewed studies of intranasal infection of cattle using different methods of virus exposure, including: (1) instillation or spraying artificially produced virus suspension into the nostrils; (2) exposure to aerosols from infectious animals through a mask; and (3) by indirect contact with infectious animals. **Table 3** presents studies that were designed to estimate infectious doses using artificially generated aerosols and animal source aerosols.

Table 3. Estimates of intranasal infectious doses of FMDv under varying experimental conditions in cattle (adapted from Seller and Gloster, 2008)

Method of exposure in cattle	Infectious Dose Range ($TCID_{50}$)	Reference
Mask with artificial aerosols generated from spinning top	12.5 to 160,288	Donaldson et al., 1987
Mask exposure to natural pig aerosols	25 to 127	Donaldson et al., 1987
Indirect contact exposure to pigs	254 to 25,403	Donaldson and Kitching (1989)

5.4 Incubation Period

The incubation period of an infectious disease is the time interval between exposure to an infective dose and the development of clinical signs. The incubation period for FMDv is known to be variable and dependent on the strain and dose of the virus, the route of transmission, the husbandry situation, and the species (Alexandersen et al., 2003b,c). It is well known that FMDv infected animals can shed the virus during the incubation period, before the first detectable clinical signs are noted. The peak of viremia can occur just before the animal breaks with clinical signs (Orsel et al., 2009). For control purposes, OIE uses 14 days as the FMDv incubation period

(OIE, 2012). Alexandersen et al., (2003c) summarized the variability observed in the incubation period of the disease as follows:

- There is a strong relationship between the dose of the virus and the length of the incubation period; for example, the higher the dose or intensity of contact between animals, the shorter the incubation period.
- For farm-to-farm spread, the incubation period ranges from 2 to 14 days for direct contact, and 4 to 14 days for airborne transmission and indirect contact routes.
- Within-farm spread is usually 2 to 14 days, but can be as short as 24 hours.
- When spread is occurring within a herd, the typical incubation period is 2 to 6 days, but can be as short as 1 day or as long as 14 days.
- The mean incubation period for cattle-to-cattle direct contact is 3.5 days, based on continuous exposure under experimental conditions (Alexandersen 2003b, 2003c).
- In cattle inoculated by the intradermolingual route, the earliest detection of FMDv in milk was 2 days post inoculation for the inoculated animals and 3 days for the direct contact animals (Reid et al., 2006).

5.5 Clinical Signs

The clinical signs of FMDv in cattle are indistinguishable from those of other vesicular diseases. The signs and severity of FMDv vary with the species of animal, the serotype, and the strain of the virus. FMDv is characterized by an acute febrile reaction (103° to 105 °F) and formation of vesicles that are found in the epithelium of the nares, lips, gums, tongue, teats, coronary bands, and interdigital spaces. The clinical signs associated with infection include lameness (usually 2-legged) manifested by foot “flicking” or treading, anorexia, depression, excessive salivation, and reluctance to move or rise. Heat and pain may be detected in the feet for 1 to 2 days before vesicular lesions appear. As the vesicles rupture, erosions are formed which can lead to prolongation of signs. The rupture of the vesicle, especially on the feet or teats, may predispose the affected areas to secondary infections, which complicate and prolong the healing process. Pregnant cows may abort and young calves may die without developing any vesicular lesions. Possible complications include temporary or permanent decreases in milk production, chronic lameness or mastitis, weight loss, and loss of condition. Milk production may never recover to pre-infection levels. Death usually occurs only in young animals as a result of multifocal myocarditis and vesicles are not always found (The Center for Food Security and Public Health, 2007; United States Animal Health Association, 2008).

6. FMD Outbreak Information

Trying to forecast the behavior of FMDv in an outbreak is challenging because each of the seven serotypes of FMDv produce clinically identical disease in livestock, but their individual epidemiological behavior is unique. This makes it impossible to predict with low uncertainty about what to expect in an outbreak, as one cannot consider it a single disease which behaves in a predetermined manner (Kitching et al., 2005). FMDv is challenging to control because (Honhold, 2011):

- Low infectious dose by inhalation
- Produced in vast quantities relative to infectious dose
- Relatively stable under many normal environmental conditions
- Many routes of infection
- Many susceptible species
- Contamination of inanimate objects (fomites)
- Human activities spreads the virus

An extensive literature review was conducted by the risk analyst to incorporate all studies and technical reports that evaluated the involvement of milk tankers in the spread of disease in past outbreaks. The conclusions from different studies were often contradictory and did not provide definitive answers. The three studies found that provided up-to-date and concise information on what is known and unknown about involvement of milk tankers, people, and vehicles in the spread of disease, and that was applicable to this risk assessment, are:

- "Involvement of Milk Tankers in the Spread of FMD in Cumbria, 2001" (Honhold et al., 2004a)
- "Procedures for Preventing the Transmission of Foot-and-Mouth Disease Virus to Pigs and Sheep by Personnel in Contact with Infected Pigs" (Amass et al., 2003a), and
- "Risk Factors for Transmission of Foot-and-Mouth Disease during an Outbreak in Southern England in 2007" (Ellis-Iversen et al., 2011).

The main points of these studies are summarized below.

6.1 Involvement of Milk Tankers in the Spread of FMD in Cumbria

The report *Involvement of Milk Tankers in the Spread of FMD in Cumbria, 2001* (Honhold et al., 2004a), was produced for the United Kingdom Department for Environment, Food, and Rural Affairs (DEFRA) and provides a comprehensive and critical review of the literature on what is known and unknown about the spread of disease and associated role of milk tankers (Honhold et al., 2004a). The key points of this report are summarized below.

“There is a significant gap in our knowledge of how the disease spreads. Before movement controls are imposed, it is clear that spread is often by the movement of infected animals and vehicles that have carried them. The spread of virus by vehicles and personnel moving between farms is accepted in the literature to be part of the epidemiology of the disease. Any vehicle visiting the farm can be involved, with milk tankers having the additional risk of carrying contaminated milk. Analysis of involvement of milk tankers and other milk collection vehicles in the spread of disease in past epidemics (1967/68) indicate the evidence was equivocal” (Dawson, 1970; Honhold et al., 2004a; Hugh-Jones, 1976).

“There are two routes by which farms became infected during the 2001 epidemic once animal movements were controlled, (1) across the fence (direct or close contact between livestock) or (2) through the farm gate (via fomites). Research by the authors and others suggests that the majority of infection risk for contiguous (adjacent farms) premises came through the farm gate, emphasizing the importance of biosecurity at this critical control point. Dairy farms should be targeted for enhanced biosecurity from the outset of any future epidemic. This enhanced biosecurity will also mitigate risk by other means of the virus entering through the gate” (Honhold et al., 2004a).

“During the 2001UK epidemic there was a clinical impression that milk tankers were linked to the spread of disease. In Cumbria, the milk tanker movements were the strongest and often the only substantial link between two infected premises (IPs). The evidence for fomite spread, and in particular the spread via vehicles and personnel is circumstantial and is based on field investigations of links between IPs, rather than experimental or controlled studies. It has been common during an epidemic for much of the spread of disease to be described as local, which in reality means that the route by which the disease entered the farm is unknown” (Honhold et al., 2004a).

“In the 2001epidemic, the initial analysis ascribed 80 percent of the IPs to local spread, which was defined as an IP occurring within 3 km of a previous IP and where more than one possible conveyor was identified, meaning a single most likely source of infection could not be identified (Gibbens et al., 2001). In the 1967-68 epidemic, 91 percent of the outbreaks were attributed to local spread (Sanson, 1994). In general there is a lack of exact understanding of how virus is transferred once on a farm. This applies to all potential vehicle and personnel sources, not only to milk tankers” (Honhold et al., 2004a).

It is generally accepted by FMD researchers that both vehicles and personnel are significant means of transferring virus between farms (a form of fomite spread) although the evidence for this is from field observations. “During the later stages of the 2001 outbreak, DEFRA staff accompanied milk tankers to observe pumping of milk and biosecurity practices. The staff noted problems of milk spillage and poor biosecurity, including significant fecal contamination of the transfer hose and tanker tires, even after cleaning and disinfection, and contamination within the inside of the tanker cab from dirty boots. The imposition of enhanced and enforced biosecurity which included DEFRA staff riding in milk tankers and the fitting of pressure washer to these

vehicles, giving drivers more opportunity to undertake efficient cleaning and disinfection, seemed to be related to the ending of the local epidemics in the areas of Thirk, Allendale and Cumbria" (Honhold et al., 2004a).

Honhold et al., (2004a) conducted a matched case control study of dairy farms and milk tanker movements from the 2001 Cumbria outbreak and compared the number of risk visits by tankers between farms which became infected (cases) and paired farms which did not (controls that remained standing until the end of the epidemic), matched by location and herd size (Honhold et al., 2004a). Based on the time of year (Feb 20th to April 20th, 2001) milk tanker would have been the most common vehicle to be moving between farms. Based on the risk visits, no differences were found between the two groups. Whether a farm become infected or not, was attributed to the differences between farms in application of biosecurity measures at the farm gate by the tanker driver and farmer. This conclusion was further supported by work which indicated that most disease transmission took place through the farm gate and that biosecurity measures differed between farms which became infected and those which did not. The study outcome indicates the importance of biosecurity in protecting farms from risk of infection during an epidemic (Honhold et al., 2004a).

6.2 Procedures for Preventing the Transmission of Foot-and-Mouth Disease Virus to Pigs and Sheep by Personnel in Contact with Infected Pigs

Amass et al., (2003a) evaluated the effectiveness of two biosecurity procedures in preventing the mechanical transmission of FMDv (O/UK/35/2001) from human investigators to sentinel pigs and sheep. Washing hands and donning clean outerwear was insufficient to prevent the mechanical transmission of FMDv to sheep, but the procedure did appear to prevent transmission to pigs. Showering and changing to clean outerwear was sufficient to prevent infection of both susceptible pigs and sheep. The results indicated that people can act as mechanical vectors of FMD virus when they move from infected to susceptible animals, and, in particular, when handling the animals by methods that would be used routinely by personnel investigating a disease outbreak.

6.3 Risk factor for Transmission of Foot-and-Mouth Disease during an Outbreak in Southern England in 2007

The 2007 United Kingdom outbreak occurred through the accidental release of FMDv at the joint facility housing Merial Animal Health's vaccine production facility and the Institute for Animal Health (IAH) diagnostic laboratory site in Pirbright, UK. Extremely wet weather with flooding of the facility grounds and area roads occurred during this time. The drainage system from one of the buildings contained cracks and allowed inadequately treated FMDv, in the effluent, to leak from beneath a manhole cover onto soils. Construction projects on the site resulted in piles of excavated soils that were driven over by vehicles. It was concluded that contaminated material from the IAH site was transported off-site via mud and contamination on truck tires. Trucks

moving soils and subsurface soils from IAH travelled on a road adjacent to the index case farm to get to the landfill. (Department for Environmental Food and Rural Affairs, 2007).

The index case farm was located 2.9 miles from the IAH site. Flooding of the road and fields was suspected to have contributed to introduction of the virus into livestock pastures from the trucks passing by the property. A tractor was driven into the flooded pasture daily to deliver feed to the livestock. The other primary case farm (reclassified as a secondary farm) experienced no flooding, but had livestock fields adjacent to a road crossing a main highway. A horse stable located next door to this farm was suspected to have produced increased road traffic and thus increased the risk of contact between the livestock and non-farm related people or materials.

A case-control study was conducted of the secondarily infected case farms (7) with 22 uninfected control farms within the Control Area to determine potential risk factors (Ellis-Iversen et al., 2011). Infection of secondary case farms appeared to be driven mainly by biosecurity and environmental risks, suggesting that both windborne spread and fomite transmission occurred. The results indicated that farms with higher biosecurity had a reduced risk of FMD infection during the outbreak. Comparison of secondarily infected case farms with control farms revealed the following:

- Secondarily infected farms were less likely than control farms to have gates/fences at the entrance to the livestock area
- Case farms experienced more unusual events, such as builders or travelers on their land during the risk period
- Case farms were also less likely to have visitor parking areas separate from the livestock areas
- All secondary case farms had roads bordering their livestock fields, a significantly higher proportion than was observed for the control farms
- Secondary case farms were located closer to the most likely primary case
- Secondary case farms had significantly more calves born in their fields than the control farms during the risk period

“During future outbreaks it may be recommended that no unnecessary vehicles enter livestock areas, as they may transport the virus attached to soil, dust, or mud particles. The presence of gates or physical barriers to livestock areas was also less likely on infected farms, which may have allowed more movement of people into livestock areas. All secondary case farms were located next to public roads, which suggested an increased risk of contact between passers-by and cattle, or splashes from vehicles reaching the animals. Moving curious young animals away from perimeter fields and avoiding outdoor calving may also reduce the rate of transmission in a population” (Ellis-Iversen et al., 2011). Stringent biosecurity and strict control of all movements on and off livestock farms are considered the best control options to date (Kitching et al., 2005).

7. Risk Assessment

7.1 Conceptual Model for Virus Transport

An exposure pathway (route of transmission) model describes the physical movement of microorganisms from a source to an endpoint that results in exposure of humans or animals to the organism. Exposure pathways are often complex and can include multiple routes of exposure including aerosolization, water, food, soil, fecal-oral, and contaminated inanimate sources. Modes of transmission of organisms can include wind, flowing water, soil movements, equipment movement, or people movement. A conceptual model was developed that integrates all of the exposure pathways for movement of FMDv from the source (infectious cows) through entry of virus onto another farm resulting in exposure of susceptible livestock through the transport of raw milk. By identifying the exposure pathways that present a risk, appropriate mitigation measures can be employed to reduce exposure to the virus.

On an infected but undetected farm, preclinical infected cows will produce infectious/contaminated milk and secretions (manure, urine, saliva) prior to the detection of disease in the herd. These secretions will be mixed into the soils and manure resulting in infectious environmental media (soils, mud, and manure slurry). The manure mixture can be transported throughout the farm by contamination of equipment (tires, loading buckets, vehicle undercarriage, etc). Farm personnel walking in animal areas, or having direct contact with livestock and equipment, will potentially spread the virus throughout buildings, on equipment surfaces, and on farm grounds. Weather events including wind, rain, and snow will spread infectious materials from their intial source areas. The daily farm activities will effectively spread the virus throughout the farm.

For the risk assessment, the sources of infectious materials were divided into two main categories: infectious milk and contaminated environmental media (manure, urine, saliva, leaked milk). Both serve as reservoirs, to further contaminate objects and people, and can lead to transport of virus from an infected farm. The pathways for movement involve multiple steps (smaller pathways within larger pathways) in the transport of the virus off-site. To avoid confusion, the OIE terminology for exposure and entry (release) are not used in this risk assessment as they have very specific meanings. Movement of FMDv occurs through multiple steps by contamination of objects and persons that transport the virus to a susceptible farm. Once on this farm, there are multiple routes for a virus to move within a site, which may lead to exposure of susceptible livestock. Below are the general sources and pathways that allow movement of virus from daily activities. Chapters 9 and 10 present an evaluation for the risk pathways.

7.1.1 Background of Dairy Activities

Things are always in motion on a dairy. Traffic includes animals, personnel, machinery, feed, manure, milk, and water (Graves and Light, 1980). Animals move to and from milking areas,

feeding areas, resting areas, treatment areas, between groups and pastures, and even on and off the farm. Personnel traffic includes those working directly with the animals via milking, observing, or treating the animals and those who are serving animals by delivering feed, removing manure, bedding stalls, and performing maintenance operations. Machinery moves around for feed handling, manure removal, and other mechanized chores. On large dairies several activities need to occur simultaneously, including milking and feeding, manure scraping, or loading groups of cows into holding areas (Graves, 1994). Non-farm personnel who may drive onto the site include feed delivery truck drivers, hoof trimmers, veterinarians, visitors, and other delivery truck drivers. Vehicles and personnel access multiple areas of the farm, including the driveway and the area around the front of the milk house.

Milk tankers usually enter a farm on the common driveway that is used by any vehicle entering or working on the premises. The tanker is parked close to the milk house, as the transfer hose that connects to the bulk tank is relatively short. Animal pens are often close to the milk house and driveway. The dairy office and milking parlor can be within feet of the milk house. Cattle may cross the driveway on some farms while moving to the milk house or other areas of the farm. Calf houses may also be close to the main driveway. **Figure 1** shows a typical dairy layout and the proximity of animal areas to the road, driveway, and the milk house. The driveway route of the tanker is indicated on the figure.

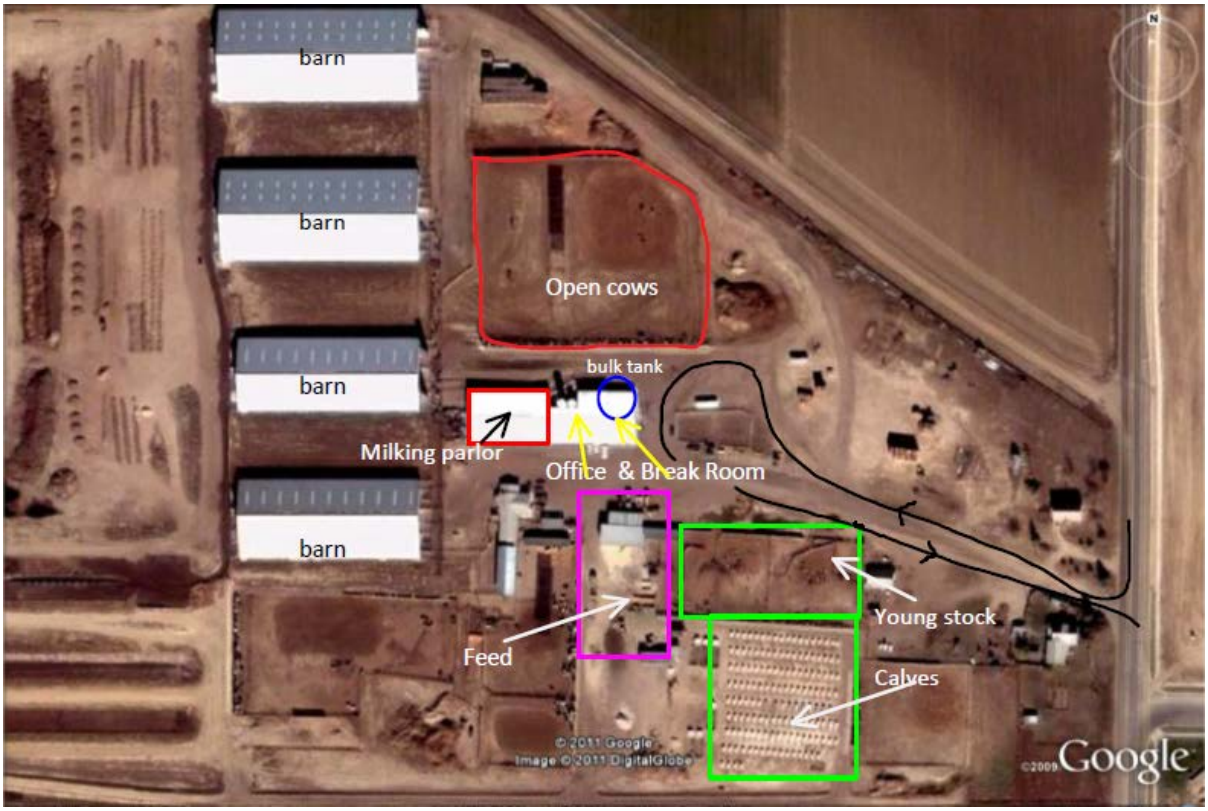

Figure 1. Typical layout on a dairy farm.

7.1.2 Daily Activities that Lead to Potential Contamination of the Tanker and Hauler

There are three routes in which milk moves from Grade A farms to the processing plant:

1. **Multiple farm pick ups:** Tanker picks up milk from multiple farms to fill tank and then travels to the processor for unloading. The truck may return to pick up milk at additional farms prior to returning to the processing plant and performaning CIP at the end of the day.
2. **Single farm pick up with multiple loads per day:** Milk is picked up from a single farm. The tanker returns to the processing plant for unloading and then travels to the next farm for another pick up. Multiple farms can be visited within a 24-hour period, with CIP only occuring at the end of the day.
3. **Single farm pick up (direct load):** Tanker is only used on one premises, as a moveable bulk tank. After the tanker is full, it is picked up by the hauler and a clean tanker is left on the farm.

Figure 2 depicts the general overview for collection of milk by a tanker. It also depicts the contact points where the tanker and hauler will become contaminated with infectious materials on an infected but undetected farm. The tanker enters a farm via the driveway, parks and conducts milk pumping activities. When traveling onto a farm, it is not uncommon for manure, mud, soil, or dust to be present in the driveway and in the area where the tanker is parked. The tanker exits the farm on the driveway and travels on the road to another farm or directly to the processing plant. Tankers may pick up milk from multiple farms prior to traveling to the processing plant to unload. Within a 24-hour period, tankers may make multiple trips to the processing plant before they undergo CIP of the internal tank. Following PMO guidance, the CIP process is required once every 24 hours. Thus a tanker could deliver and unload infected milk and travel to other farms prior to any cleaning and disinfection. External cleaning of the tanker is left up to the discretion of the company.

At the dairy farm, prior to pumping raw milk into the tanker the the hauler conducts many activities including the collection of milk samples for quality control measurements. The hauler pulls the transfer hose through the port door to connect the bulk milk tank to the tanker. He may climb onto the top of the tanker to open the vents, move within and out of the milk house multiple times, and walk to and from the tanker cab. He may also walk into the office, milking parlor, or other areas of the farm and interact with farm employees. During coupling and uncoupling of the transfer hose, milk may be spilled onto the milk house floor, as well as outside of the milk house. Spillage of milk on the floor is not uncommon and it is cleaned up at the end of pumping. There is some loss of milk at each unhooking of the transfer hose ranging from a couple of cups to a gallon. Five to ten gallons of milk can be left in the transfer hose after uncoupling from the bulk tank (Gibson, 2011). For truck mounted transfer hoses, when pumping is complete, the end of the transfer hose is capped and the hose is moved through the port door and rolled up and placed within the storage compartment of the tanker.

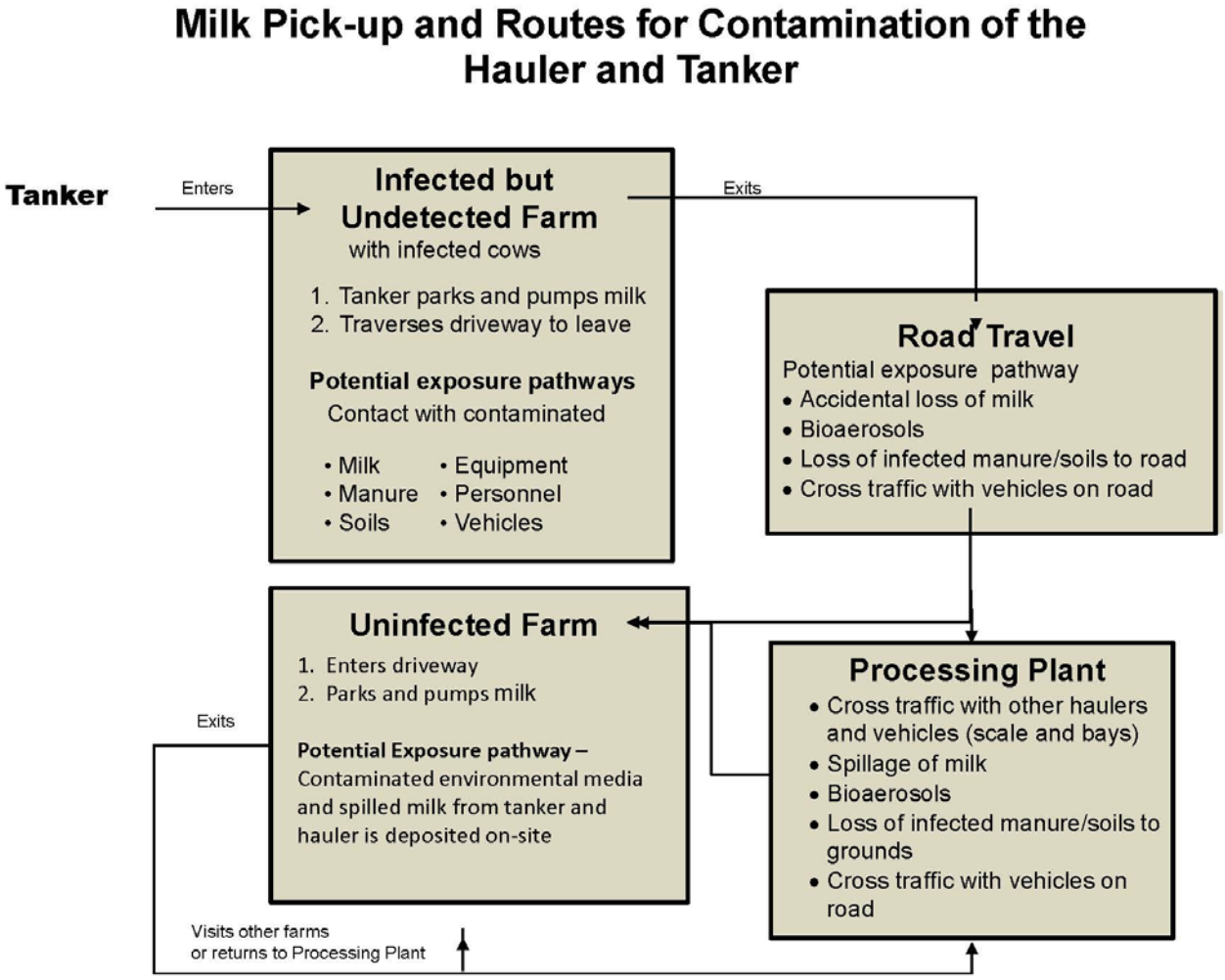

Figure 2. General tanker pick up route and points of contact for virus exposure.

During pumping activities, there are multiple sources and routes for infectious materials to come into contact with the hauler's boots, clothing, and hands including contaminated soils outside of the milk house and fomites within the milk house. It is not uncommon for haulers to get manure and spilled milk on their clothing on a daily basis (Gibson, 2011). The hauler has contact with infectious milk during coupling and uncoupling of the transfer hose. External contamination of the transfer hose can occur both outside of and within the milk house by contaminated soils and infectious milk. Leakage of milk may occur within the storage compartments of the tanker and milk may also be spilled on the ground, which the hauler may walk through. Farm personnel may also walk through the milk house during pumping activities through spilled milk on the floor and track it to other areas of the farm. Upon completion of pumping activities, the tanker and hauler exit the farm driving through potentially contaminated environmental media.

For multiple farm pick-up routes, the newly contaminated tanker and hauler will enter an uninfected farm to pick up milk. Contamination of the tanker, transfer hose, hauler, or storage compartments now serve as a source of infectious virus that can be released onto the farm grounds and facilities through the hauler's activities. The most likely route for spread of virus is through the farm gate via fomites. There is outbreak evidence that virus was carried and dispersed by truck tires during the 2007 outbreak (Ellis-Iversen et al., 2011). **Figure 3** presents the interaction between farm activities and the tanker and hauler that could lead to transfer of virus to the uninfected farm. This diagram is applicable to both how a farm could contaminate a tanker/hauler with virus and how the tanker/hauler can subsequently expose an uninfected farm.

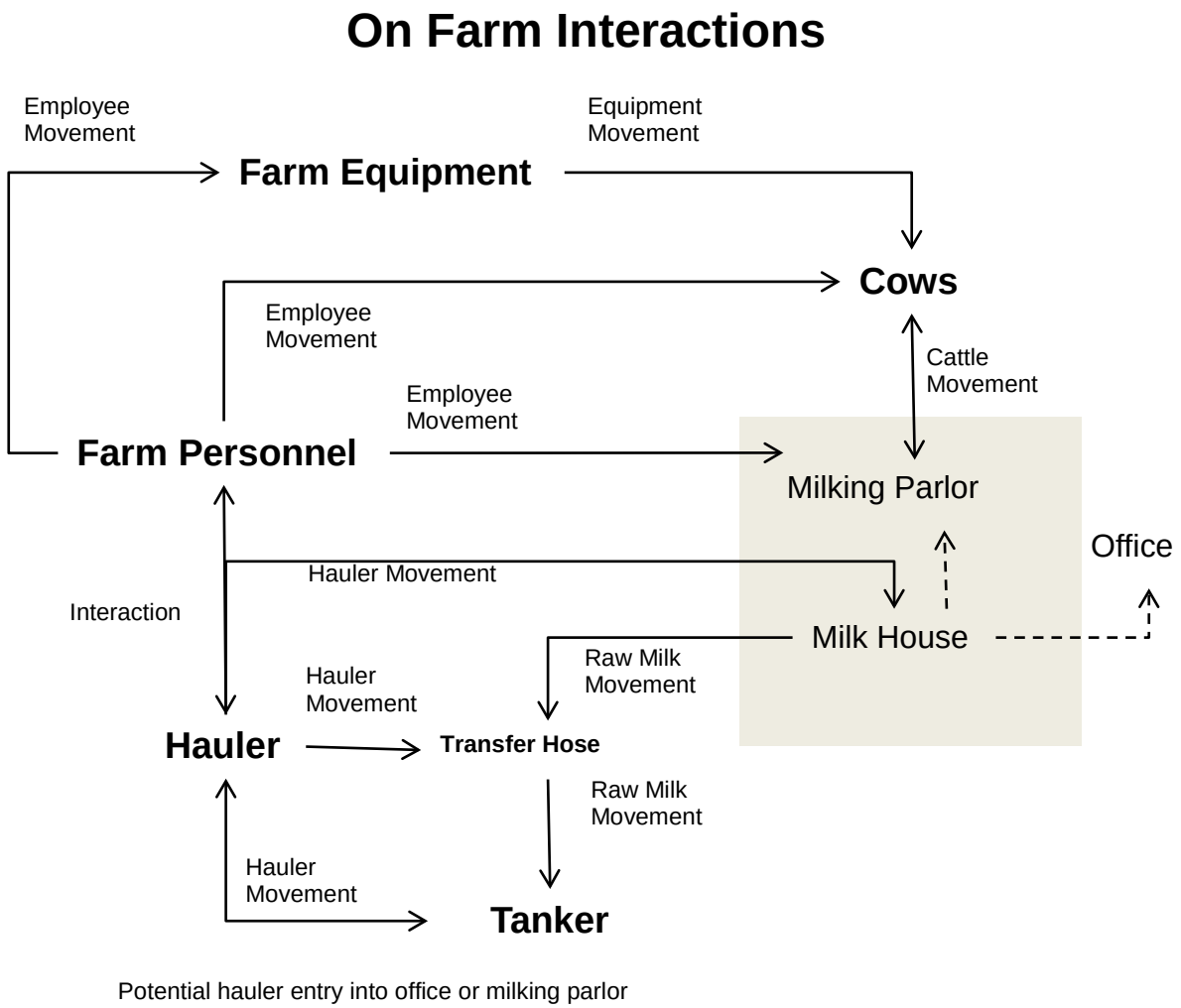

Figure 3. On farm interactions leading to virus transfer.

At the susceptible/uninfected farm, the hauler may transfer contamination from his boots, clothing, hands, and equipment to the parking area outside of the milk house and the floor and equipment surfaces within the milk house. If they walk into other parts of the building, such as the office and milking parlor, contaminated fomites can be left in these areas. Environmental contamination that is present on external surfaces of the transfer hose can serve as a source of infectious virus both outside on the ground and inside on the milk house floor. Residual milk that is left in the truck mounted transfer hose—or spilt milk in the storage compartments—can leak onto the ground outside and inside of the milk house. External contamination on the tanker can be deposited on the ground dependent on the strength of adherence and weather events such as rain, water splashing, or wind. There is a theoretical risk of producing bioaerosols that escape the tanker through the manhole cover during milk pumping, which is presented in Chapter 9.

Observation of milk pumping activities indicate that farm personnel routinely walk through the milk house directly into the milking parlor or the office. It is not uncommon to have water or spilled milk on the floor of the milk house or adjacent rooms from farm activities such as collection of raw milk for calf feeding or cleaning activities. Large amounts of mud and manure were observed on tractor tires and associated equipment. These examples are not unusual and are representative of the challenges to prevent transfer of virus in either direction.

How does infectious virus deposited on the uninfected farm potentially come into contact with susceptible livestock? As stated in the previous section, there is a lack of exact understanding of how virus is transferred once on a farm. This applies to all potential vehicles, personnel, and visitors, not only to milk tankers (Honhold et al., 2004a). **Figure 4** provides the pathway overview of the potential routes for movement of virus from the intial sources on an infected but undetected farm which could result in exposure of livestock on a susceptible farm. The last step of the diagram lists potential routes by which cattle may become exposed to virus after the contaminated tanker and hauler visits the farm. The main routes for exposure of livestock to virus will be through contamination of soils and milk house surfaces which leads to aerosolization of virus associated particulates by wind and cleaning activities, soil and manure runoff, and mechanical tranfer by people, equipment, and vehicles into the milking parlor and pens. Bioaerosols from pumping of milk are another route that was considered.

Another pathway for transfer of virus occurs at the processing plant or transfer station. **Figure 5** presents the interactions that occur on the processing plant grounds. The most likely scenarios for transferring virus is by loss of contaminated materials on the tires and undercarriage to the ground. This leads to cross-contamination of other tankers in the driveways, weight scale, storage, or unloading areas. The hauler can potentially carry contamination on his boots and clothing into any plant areas that they visit. As stated previously in the on-farm scenarios, their interactions with other haulers – or processing plant staff – could lead to fomite transfer and a newly contaminated hauler.

The performance of CIP at the plant will directly address sources of infectious milk within the tanker, the residual milk in the transfer hose, and spilled milk in the tanker compartments. CIP will not address external contamination of the tanker because external washing of the tanker is not conducted in tandem with the CIP process. If CIP is not conducted after unloading, milk will remain in the transfer hose and spilled milk within storage compartments. Direct load trailers are expected to undergo CIP prior to a subsequent use. This would fall under the regulations pertaining to cleaning the bulk tank after each use. There are two CIP-related scenarios that affect the transport of infectious materials between farms:

1. Multiple farm pick ups with no CIP between farms or loads
2. Single farm pick up with no CIP between loads

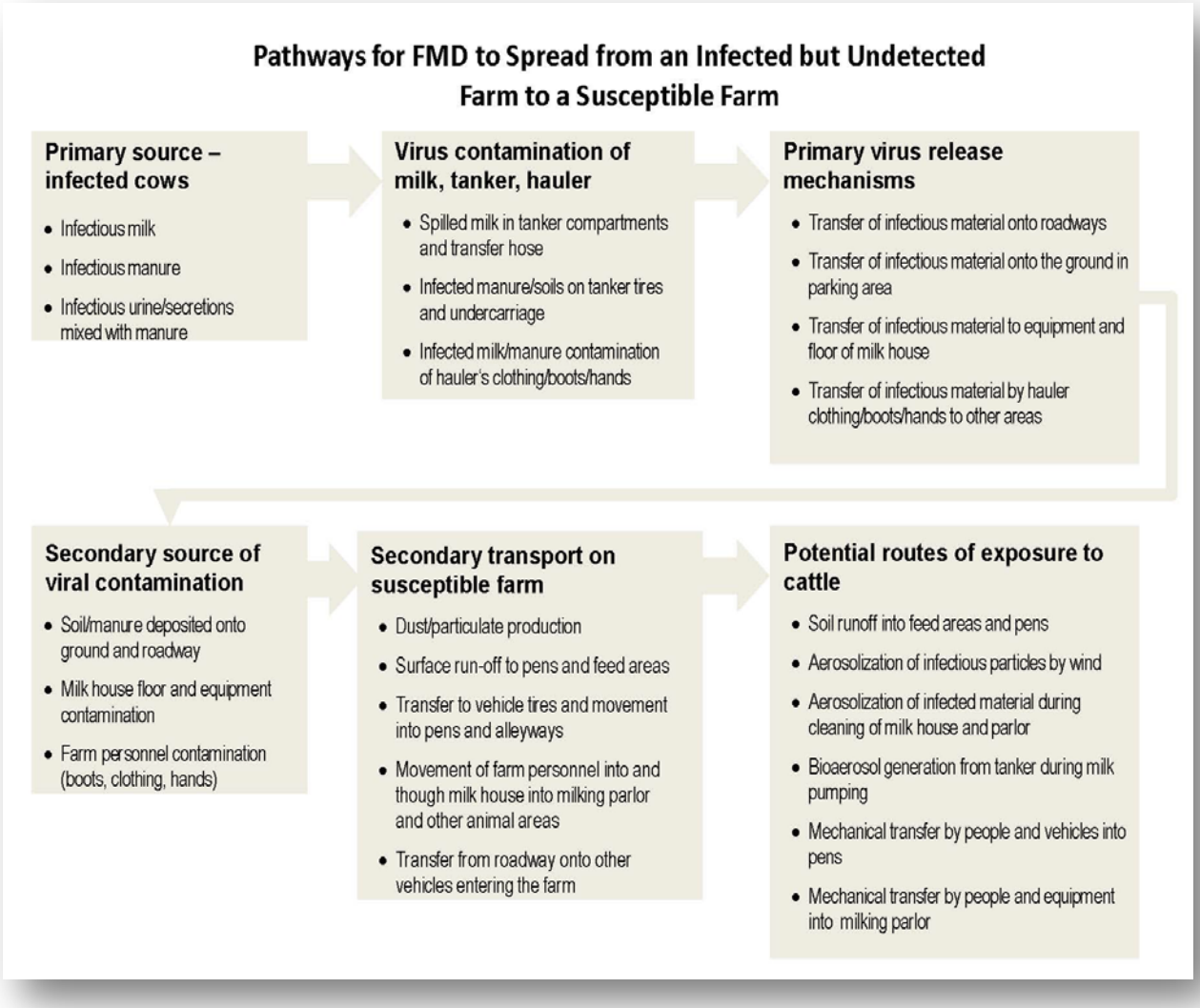

Figure 4. Pathway from primary viral sources leading to release and exposure of susceptible livestock.

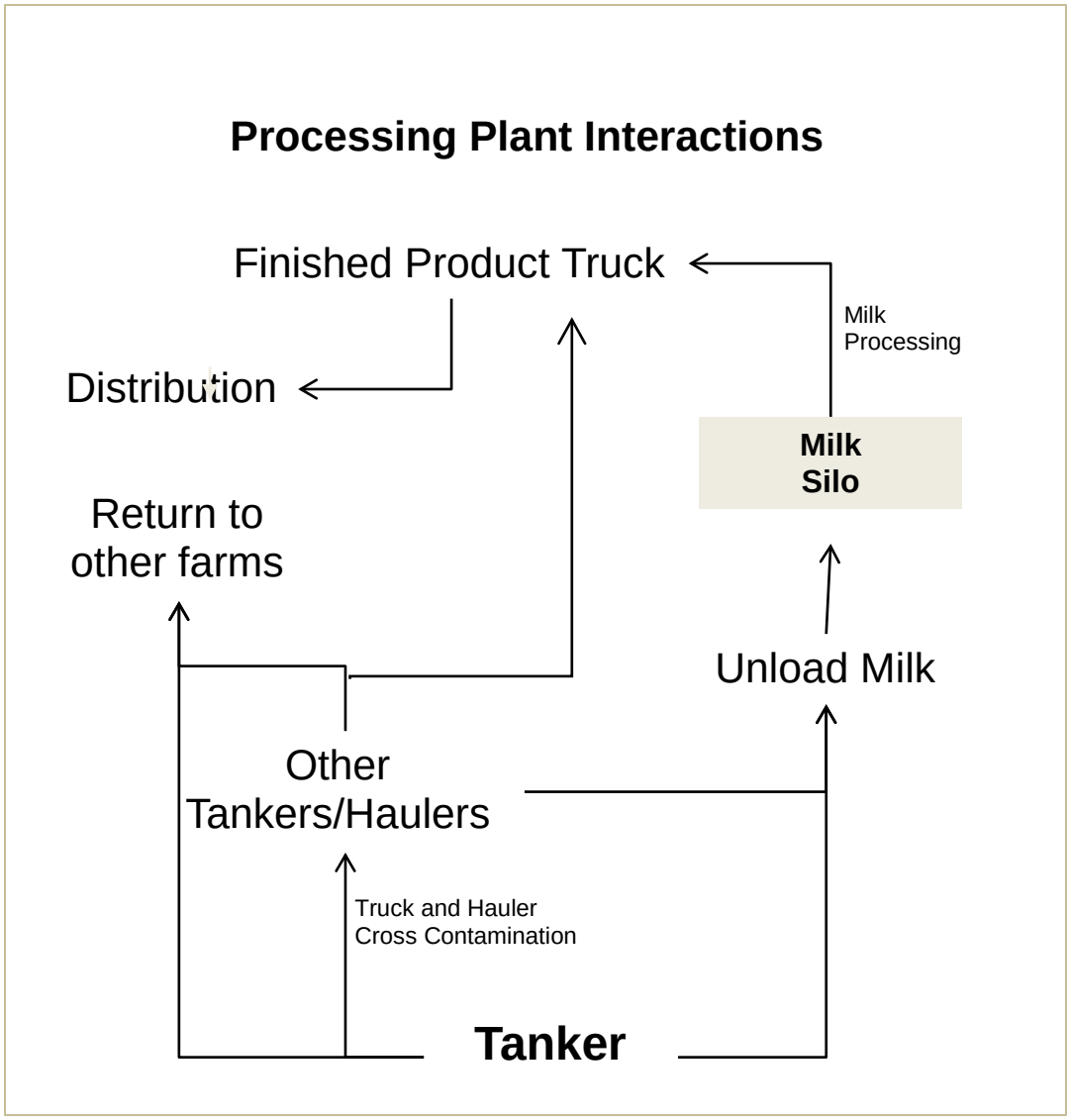

Figure 5. Processing plant interactions.

Chapter 9 and 10 present the evaluation of risk for each of the scenarios within the Infectious milk pathway (Chapter 9) and the Contaminated Environmental media pathway (Chapter 10).

7.2 Chapter Conclusion

Analysis of historical outbreak studies have shown that once control measures are in-place, the predominant route for virus entry onto a farm is through the farm gate (via fomites). Once the virus is on a farm, the routes of spread of virus are not well known. During the 2001 outbreak in Cumbria, United Kingdom, the difference in whether a farm became infected or not, was directly related to applied biosecurity measures at the farm gate by the tanker driver and the farmer. In the 2007 United Kingdom outbreak, muddy construction vehicles with virus contamination on the tires and undercarriage were implicated as the source of viral spread. These vehicles did not enter the index case farm, but drove on roads adjacent to the farm. Research has shown that

people can serve as a source for fomites and spread the disease, even after hand washing and changing outer clothing.

The conceptual model presents the main exposure points for the hauler/driver, tanker, and transfer hose to become contaminated with FMDv and serve as a resource for the transport of infectious material. On an infected but undetected farm, the infectious materials of concern are infectious milk and contaminated environmental media. The most plausible pathways for the transport of virus to an uninfected farm are through contamination of: (1) driver's boots, clothing, and hands by infected environmental media and spilled milk; (2) tanker's tires and undercarriage with infected environmental media; and (3) truck mounted transfer hose or farm based transfer hose with infected environmental media and spilled milk, including residual milk within the hose.

During over-the-road transport to another farm or to the processing plant, the accidental loss of milk leading to spillage on the road, or generation of bioaerosols, is another potential route of exposure for cross-contamination of other vehicles. The potential to generate bioaerosols during pumping at a subsequent farm is also a route for exposure. The performance of CIP – or lack of implementation of CIP at the processing plant – will affect milk residues within the tanker, spilled milk in the tanker compartments, residual milk left in the transfer hose, and external contamination on the transfer hose. It does not address external contamination on the tanker. Washing of the tanker is required to remove contamination present on the tires, wheel wells, undercarriage, and sides of the tanker—which is not part of the CIP process. The transfer of contamination to uninfected farms results in fomite contamination of farm personnel, vehicles, and grounds that results in infection of susceptible livestock through aerosols, soil run-off, and mechanical transfer by people, equipment, and vehicles.

8. Disease Spread Model

This section presents the background information, studies, parameters, and description of the development and application of a disease spread model used to estimate concentrations of infectious virus that could be present in a bulk milk tank on an infected but undetected farm. Three farm sizes of 100-, 500-, and 1,000-cow dairies were evaluated. The goal of this section was to estimate concentrations of virus over time that could be present in a tanker leaving an infected but undetected farm.

8.1 Background: Behavior of FMDv in Milk

FMDv spreads very efficiently when first introduced on a farm, and the prevalence on any given farm is likely to be rather high before disease is detected, in particular if there is free contact among animals (Alexandersen, 2005). Dairy cattle have the highest relative risk among all livestock species and production types of becoming infected based on previous outbreak experience and spatial modeling. Only a few dairy herds may be affected at the time of the initial report, but the prevalence of FMD infection may vary from 10 to 90 percent among dairy cattle in any individual herd. The risk of spread by milk is mainly in the early phases of infection—before disease is officially reported and mitigation procedures are put into place (Alexandersen, 2005).

Virus is excreted in significant titers in the milk from 2 to 4 days before clinical signs appear and through 4 to 5 days of the clinical phase, in a pattern that largely mirrors the viremia profile. Reduction in milk production has been reported in the literature, but has not been a consistent feature of all outbreaks. After the high level of excretion during the acute phase, very low levels of FMDv have been reported to be excreted in milk for up to 3 weeks or more (Burrows et al., 1971). Large amounts of virus are excreted in vesicular fluid, in desquamated vesicular epithelium, and in cattle and in saliva (Cottral, 1969; Hyslop, 1965; Scott et al., 1966). There is also excretion—but to a much lesser extent—in feces and urine (Burrows, 1968; Garland, 1974; Parker, 1971). A sharp decline in viral excretion and load occurs around day 4 to 5 of clinical disease, when a significant circulating antibody response is detectable.

During the 1981 Isle of Wight, United Kingdom outbreak, FMDv titers in milk samples from 6 clinically normal cows, on a farm with FMD positive animals, ranged from $10^{0.7}$ to $10^{6.6}$ $TCID_{50}$/ml. Bulk milk tank samples collected from 32 cows reported an FMDv titer of $10^{2.2}$ $TCID_{50/}$ml (Donaldson et al., 1982). Milk titers in certain situations may be high (e.g., $10^{5.5}$ $TCID_{50}$/ml and 10^4 MID, [mouse infectious dose equivalent to approximately 10^5 $TCID_{50}$/ml]) which was reported for a milk churn, milk tanker, and a retail bottle of milk (Anonymous, 1968; Burrows et al., 1971; Hedger and Dawson, 1970). The risk of animal's becoming infected if directly fed such milk is substantial, as the dose required to infect by the oral route is only 10^4 to 10^6 $TCID_{50}$.

8.2 Stochastic Disease Transmission Model

The objective of the modeling was to simulate the spread of FMDv within a herd and to estimate the number of cows in various disease states at each time period. The disease states include susceptible (S), latent (L), preclinically infectious (PI), clinically infectious (CI), and recovered (R) (Perez et al., 2004). The model updates the number of cows in each of the disease state every 6 hours, which increases the accuracy of detection. The uncertainties in input variables and the variability associated with the course of infection in cattle, and spread within the herd, are considered in the model. Appendix C presents the assumptions, definitions, and background information used in the disease transmission model and the details for transitions between disease states.

8.2.1 Simulation of FMDv Titer in a Bulk-Milk Tank

The model simulates the FMDv titer in a bulk-milk tank updating it at each time step. The following factors are considered in estimating the FMDv titer in the bulk-milk tank:

- The estimated number of preclinically and clinically infectious cows in each time period
- The uncertainty and variability in the FMDv titer of milk from preclinically and clinically infectious cows
- The frequency at which the milk from the bulk tank is transported to the processing plant (once per day for 500- and 1,000-head herds and every other day for 100-head herds)

The following simplifying assumptions were used in implementing this model:

- Additional milk is added to the bulk milk titer in each time period
- Milk production rate is a constant for all cows in the herd
- The bulk-tank milk is emptied just prior to detection of disease in the herd on any day

The model calculates the mean FMDv titer (PFU/ml) in milk added to the bulk tank, based on the number of preclinically infectious and clinically infectious cows in that time step. The model updates the FMDv titer and amount of milk in the tank, while considering the volume and viral titer of the milk already present in the tank. The volume of milk present in the tanker is reset to zero in the time period when the bulk tank is emptied and milk transported to a processing plant. For smaller farms, which have every other day pick up, the dilution of FMDv titers in milk–due to mixing with milk collected over two days–is considered by the model. The model results for the milk titer in the bulk tank are presented in units of $\log_{10}$ PFU/ml.

The model assumes that all of the milk from the bulk tank is pumped into the tanker and is present in the tanker at the same concentration as the bulk tank. If additional farm pick-ups occur after collection of contaminated milk from an infected but undetected farm, the milk in the tanker

will be diluted by uninfected milk. In this risk assessment, we are evaluating a worst-case scenario in which milk is picked up from an infected farm and then travels directly to other farms or the processing plant. The next farm visited after collection of infectious milk, is the farm that is most at risk. The model does not address all situations that could arise, but addresses pathways that present the highest risk for transmission of disease. The milk from an infected but undetected farm that contaminates tanker equipment and surfaces, clothing, and environmental media with virus fomites will not be significantly diluted by collection of additional milk.

8.2.2 Detection of Clinically Infected Cows

The detection module estimates the time (days) to detect FMDv infection in the herd based on heightened active observational surveillance for clinically infectious cows using three different surveillance levels. The model "checks the data," or applies specific detection mechanisms, in a specified time step, as determined by the user. The model assumes that personnel observe all of the cattle visually the equivalent of once per day for clinical signs of disease. Although cows are routinely milked two or more times per day, it was assumed that each cow would receive the equivalent of an overall body visual observation for clinical signs between all daily milking activities. In the model, the "check step" of the data and subsequent detection of disease occurs a short time after the bulk tank has been emptied and the milk tanker is on the way to processing. This is a conservative assumption as milk will have been collected in the bulk tank in the last 4 time periods (24 hours) reflecting maximal contributions from all cows prior to detection of disease. The model surveillance mechanism detects disease if the percentage of clinically infectious cows is greater than an absolute percentage chosen by the user.

Three absolute percentage surveillance levels of 1, 5, or 10 percent of the herd showing clinical signs at the time disease is detected were chosen for evaluation. For example, in a 1,000-head herd, this would mean that 10, 50, and 100 animals, respectively, would be showing clinical signs at the time disease is first detected. The surveillance values are based on expert opinion and discussions with dairy owners. Outputs of the surveillance model includes the number of preclinical and clinically infectious cows, the bulk milk titer in each time step, the time of detection, as well as the time to first detect clinical disease.

Survey data from the USDA National Animal Health Monitoring System (NAHMS) on the average percentage of ill cattle that occur on a farm, prior to the owner contacting the veterinarian, are higher than the surveillance levels used in this risk assessment (**Table 4)**. During an FMDv outbreak, active observational surveillance and heightened awareness for clinical signs of FMD disease would be expected to decrease the time (and number of ill animals) to report disease to authorities.

Table 4. The operation average percentage of change at which time a veterinarian would be contacted for assistance, by potential problem sign and herd size (NAHMS, 2007).

	Herd Size (Number of cows)							
	Small (<100)		Medium (100-499)		Large (500+)		All Operations	
Potential Problem Sign	Pct.	Stnd. Error	Pct.	Stnd. Error	Pct.	Stnd. Error	Pct.	Stnd. Error
Decline in total milk production	22.3	1.2	18.0	1.1	12.9	1.2	20.6	0.9
Milk cows exhibiting fever within a short time period	10.7	1.2	7.3	0.9	6.0	1.8	9.6	0.9
Milk cows dying within a short time period	6.8	1.1	3.2	0.7	4.2	1.9	5.8	0.8
Milk cows aborting within a short time period	8.1	1.1	3.9	0.7	4.6	1.8	6.8	0.8

8.3 Disease State Parameters used in the SLIR Model

The parameters used in the model for simulation of disease spread on a farm are presented in **Table 5**. Three herd sizes were evaluated: 100-, 500-, and 1,000-cow dairies. Milk production for a cow ranges from 60 to 75 lbs/day (J. Lombard, personal communication). For this analysis, 60 lbs/day is used in the model. Bulk-milk tank emptying frequency was set daily for the farm sizes of 500 and 1,000 cows and every other day for farm size of 100.

Table 5. Model parameters used in the evaluation of milk titers in bulk tanks.

Variable	Input Distribution or Value
Latent Period	Exponential 0.709783 (mean of 33.72 hour, SD of 34.17 hours)
Preclinical Period	Lognormal mean = 0.862, SD=0.774 (mean of 76.69 hours, SD of 70.39 hours)
Clinical Period	Gamma, shape = 4.7518, scale = 0.7364 (mean 83.9 hours, SD of 38.52 hours)
Farm Size	100, 500, and 1,000 head
Adequate Contact Rate	52 to 216 contacts/day
Individual Animal Milk Production	23.38 L/day (60 lbs/day)
Preclinical Milk Titer*	1.12 $\log_{10}$PFU/ml (0.05-3.46 90% PI)
Clinical Milk Titer*	2.21 $\log_{10}$PFU/ml (0-5.10 90% PI)
Milk Emptying Frequency	1x/day for 500 and 1,000 head farm and every other day for 100 cow herd
Detection Method	1% of herd, capped at 10 cows

* Milk titer data developed through simulation step for custom distribution (see Appendix C)

The background information on development of the disease state durations, contact rates, detection method, and milk titer values used in the model are discussed in the sections below.

8.3.1 Disease State Durations

Estimates of animal level disease stage durations (latent, preclinical, clinical, and recovered) were obtained from recently developed parameters used in the NAADSM (North American Animal Disease Spread Model) (USDA, 2012). The parameters were developed by evaluating animal level data from published studies which involved experimental infection of cattle with FMDv (Aggarwal et al., 2002; Blackwell et al., 1982, Burrows et al., 1971; Burrows et al., 1981; Gomes et al., 1997; Orsel et al., 2007; and Sutmoller and McVicar, 1976). These data were used to develop animal-level disease state distributions by re-sampling the data and generating best-fit distribution curves using Microsoft Excel®, Microsoft Excel Solver®, @Risk, and SAS® 9.2. The resulting distributions were reviewed for epidemiological application by analytical epidemiologists at USDA-APHIS-VS-CEAH. The distributions were developed to match the shape, mean values, and standard deviations of the data. Nonparametric testing using SAS® 9.2 demonstrated equivalence of the developed distributions with collected data. The nonparametric tests included: Wilcoxon test, Kruskal-Wallis, median scores, Van der Waerden, Savage, Kolmogorov-Smirnov, and Kuiper.

8.3.2 Contact Rate

The contact rate was based on a distribution of values ranging from 52 to 216 contacts/day. These values were derived by (Carpenter et al., 2004). By 9 days after the first animal (index case) became infected 50 percent of an intensively managed 1,000-cow dairy herd would likely be infected with FMDv (referred to as the cumulative infection density, CID_{50}). The parameter *k* is defined as the number of adequate contacts made by an individual animal sufficient to cause disease transmission in a specified time period. The number of adequate contacts necessary to achieve a CID_{50} by 9 days was identified as the value of *k* that generated a CID_{50} of 9, using Solver (an @Risk add-in). For sensitivity analysis, *k* values that generated a CID_{50} for 8 and 10 days were also selected. This approach generated *k* values of 0.57, 0.91, and 2.25 contacts per hour. The value of 2.25 contacts/hour was chosen for the lower bound number, based on expert opinion from dairymen and personal observation of dairy cattle interactions. This value was rounded to 13 contacts/6 hour period for use in the SLIR model.

The upper bound value of 9 contacts/hour is also from work by (Carpenter et al., 2004). The authors subjectively estimated the contact rate for a typical intensively managed 1,000-cow dairy herd based on expert opinion. The estimate assumed 100-120 cows per corral and animal-to-animal contact that was sufficiently close to be reasonably certain that FMDv would be transmitted from an infectious animal to a susceptible animals by nose to nose or nose to vulva contact. Contacts between cows with behavioral signs of estrus, as well as between bulls and cows throughout the estrous cycle, and contacts made during the twice-daily gathering and crowding of lactating cows before milking were included. The estimate also considered

observations of physical contact across fences and across water troughs shared by corrals and physical contact made with adjacent cows while eating or locked in stanchions (Carpenter et al., 2004).

8.3.3 Studies used to Develop Milk Titer Values in the SLIR Model

Five studies were found that contained individual cow FMDv milk titer data for preclinical and clinical stages of disease. Data for in-contact dairy cow exposure were used in this analysis and inoculated animal data were excluded. Four of the studies were experimental studies, while the fifth study contained outbreak information from the Isle of Wight, United Kingdom outbreak. The studies used for estimating preclinical and clinical milk titer parameters were:

- Concentrations of FMDv in milk of cows infected under simulated field conditions Blackwell et al., (1982)
- Use of automated Rt-PCR to detect FMDv in milk and utility of automated real-time RT-PCR for the detection of foot-and mouth disease virus excreted in milk (Reid et al., 2004)
- The growth and persistence of FMDv in the bovine mammary gland Burrows, et al., (1971)
- The effect of vaccination on FMD disease virus transmission among dairy cows Orsel et al., (2007)
- Use of prediction models to forecast and analyze airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey, and the Isle of Wight in 1981 Donaldson et al., (1982)

8.3.3.1 How the Data was Evaluated

Data from all studies were categorized into preclinical and clinical stages of disease. Non-detection of virus data were assigned a value of zero. The start of the preclinical stage was determined by the first detection of virus in body fluids (nasal swabs or esophageal/pharyngeal fluids) in all studies. The start of the clinical phase was based on the report of the first clinical signs of disease consistent with FMD. Brief overviews of the experimental studies are presented in Appendix D.

The FMDv milk titers for preclinically infectious cows and clinically infectious cows were modeled separately. For preclinically infectious cows, a smaller dataset of 47 data points was available. The uncertainty associated with the mean FMD milk (1.12 log PFU/ml) titer for preclinically infectious cows was simulated, as well as the variability associated with the milk titers among different cows (see Appendix D for details). For clinically infectious cows, 97 data points on FMDv titers in milk were available, the uncertainty regarding the mean log milk titer (2.25 log PFU/ml) was not considered due to the large data size. The variability in the milk titer among individual cows was modeled using a Weibull distribution as detailed in Appendix D.

The upper and lower bound values for the preclinical and clinical titers were used in the SLIR model. During model runs, random values were selected from this range to model milk titer during the preclinical and clinical phases. The data was capped at $\log_{10}$ 5.5 to prevent large outlier values from skewing the data on the high end.

8.4 Simulation Results

The simulation model was implemented using Visual Basic.net®, R®, and EXCEL® software. Model simulations were run for 6,000 iterations for herd sizes of 100, 500, and 1,000 cows to produce the number of cows in each disease state and the milk titers at the time of detection. The mean value for all iterations combined was used to generate the SLIR graph.

The results of the SLIR simulation for a 1,000-head dairy herd are presented in **Figure 6.** Prior to 5 days post infection, note the substantial increase in latent and preclinically infected animals on a day-by-day basis, well before the first clinical cases are observed. On average, the majority of the herd (>50 percent) is expected to become infected (latent and preclinical) before there is a noticeable increase in the number of clinically infectious cows. Using the surveillance levels of 1, 5, and 10 percent of the herd showing clinical signs on the day disease is first detected, the model indicates that it will require between 2 and 9 days (5^{th} and 95^{th} percentile levels) to detect disease on a farm for all farms sizes and detection levels combined.

Table 6 presents the number of days to detect disease for each farm size (mean, 5^{th}, and 95th percentile values) by the surveillance level. On average, disease will first be detected 4 to 6 days post-infection of the herd. In the model, disease is only "checked" or detected once per day on a farm. The averaged time to detect disease is on day 5 for all farm sizes at the 5 and 10 percent surveillance level. This indicates that within 24 hours or less, the number of clinically infectious animals could double in number, due to the rapid spread of disease. It is expected that disease will be detected when the number of clinical animals is within the 5 to 10 percent surveillance range.

The mean log titer of FMDv in the bulk-milk tank on the day of detection ranges from 2.64 (100 head, 1 percent level) to 3.60 (1,000 head, 10 percent level) $\log_{10}$ PFU/ml for all 3 dairy sizes and detection levels considered (**Table 7**). The 5^{th} to 95^{th} percentile values range from 0.67 to 3.99 $\log_{10}$ PFU/ml for all farms and surveillance levels combined. The mean log FMD titer is not significantly different between the 5 and 10 percent detection levels, which again reflects the rapid spread of disease in the herd and similar total numbers of infectious cows (preclinical and clinical) at this time.

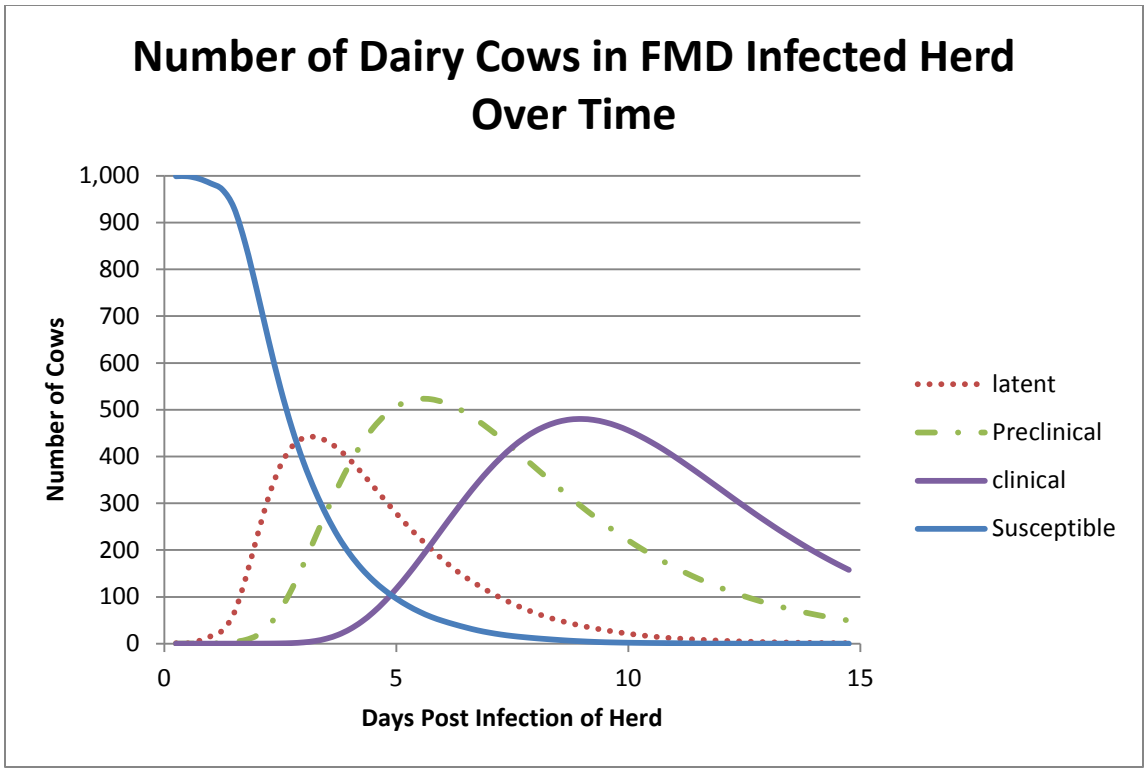

Figure 6. Mean number of cows in various disease states by days post infection in a 1,000-cow herd.

Table 6. Number of days to detect disease using a detection level of 1, 5, and 10 percent of the herd showing clinical signs.

Days to Detect Disease Post Infection (mean, 5th, and 95th percentile values)	100-cow Herd	500-cow Herd	1,000-cow Herd
1% detection level	3.9 (2-7)	4.6 (3-8)	4.8 (3-8)
5% detection level	4.8 (3-8)	5.2 (4-8)	5.4 (4-8)
10% detection level	5.2 (4-8)	5.7 (4-9)	5.9 (4-9)

Table 7. FMDv titer in the bulk tank on the day disease is detected by dairy size and detection level.

FMDv Titer in Bulk Milk Tank $\log_{10}$ PFU/ml (mean, 5th and 95th percentiles)	100-cow Herd	500-cow Herd	1,000-cow Herd
1% detection level	2.64 (0.67-3.70)	3.08 (2.22-3.73)	3.14 (2.33-3.75)
5% detection level	3.26 (2.33-3.86)	3.43 (2.86-3.90)	3.44 (2.88-3.91)
10% detection level	3.47 (2.83-3.96)	3.59 (3.12-3.97)	3.60 (3.15-3.99)

Figure 7 presents the graph of the mean log FMD titer and the 5th and 95th percentiles values by day for a 1,000-head dairy using a 5 percent surveillance level. There is significant variation in the milk titer during the initial 5 days post-infection, as indicated by the wide interval between the 5th and 95th percentile values on these days. This variation is due to the inherent differences or variability between farms (iterations) during the early stages of disease spread. The titer starts to reach a plateau around day 5, the average day to detect disease. A slight dip in the milk titer occurs each time the milk tank is emptied. After 6 days, there is oscillation in the milk titer as the number of cows transitioning to the recovered phase increases and the milk titers start to decrease.

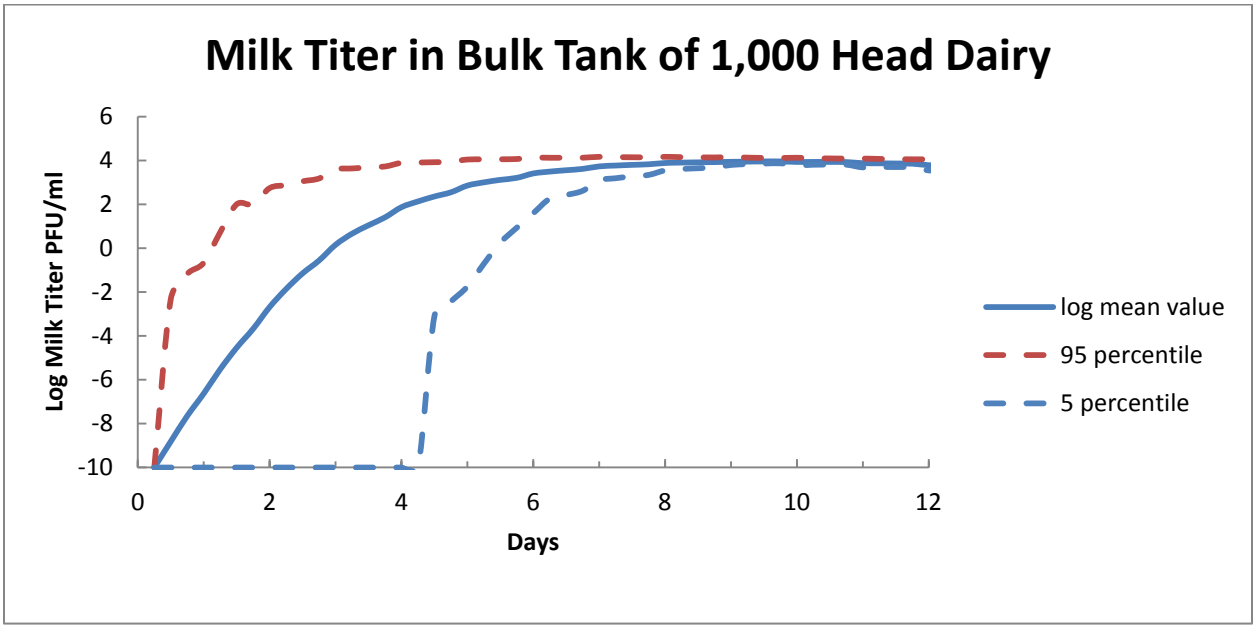

Figure 7. Variation in FMDv titer (log PFU/ml) in a bulk milk tank by day's post- infection of a 1,000-cow milking herd.

Table 8 presents the numbers of preclinically and clinically infectious cows on the day disease is detected for each herd size and surveillance level. These surveillance levels indicate that at least 1, 5, or 10 (100-head herd), 5, 25, or 50 (500-head herd) and 10, 50, or 100 (1,000-head herd) animals are clinically ill on the day of detection for the 1, 5, and 10 percent levels respectively. In the results table, when the number of clinical animals is significantly higher than the absolute percentage cut-off value listed above, it indicates the number of clinical animals increased rapidly within the previous time period since the last daily detection "check." The data indicates that 50 to 60 percent of the herd will be infectious and shedding virus by the time disease is detected. In general, over 50 percent of the cows in a herd may be in the preclinical stage of disease at the time disease is detected.

Table 8. Number of preclinical and clinically infectious cows on the day of detection.

Parameter	100-cow Herd		500-cow Herd		1,000-cow Herd	
Number of cows (mean, 5th and 95th percentile)	**Preclinical**	**Clinical**	**Preclinical**	**Clinical**	**Preclinical**	**Clinical**
1% detection level	51 (21-69)	4 (1-10)	281 (211-330)	20 (5-47)	573 (448-655)	43 (11-90)
5% detection level	63 (54-72)	12 (5-21)	320 (298-339)	57 (27-95)	640 (605-670)	113 (54-190)
10% detection level	63 (55-71)	18 (10-28)	318 (296-339)	90 (53-134)	634 (598-667)	183 (106-265)

Box plots of the percentage of infectious cows (preclinical and clinical) at the time of detection using the most conservative surveillance level of 1 percent for the 3 farm sizes are presented in **Figure 8**. Approximately 62 percent of the herd is infectious at detection; there is greater variability when the herd size is smaller. This result is expected for a smaller herd, as the variation would be averaged out in a larger sized herd within the 6,000 simulation iterations.

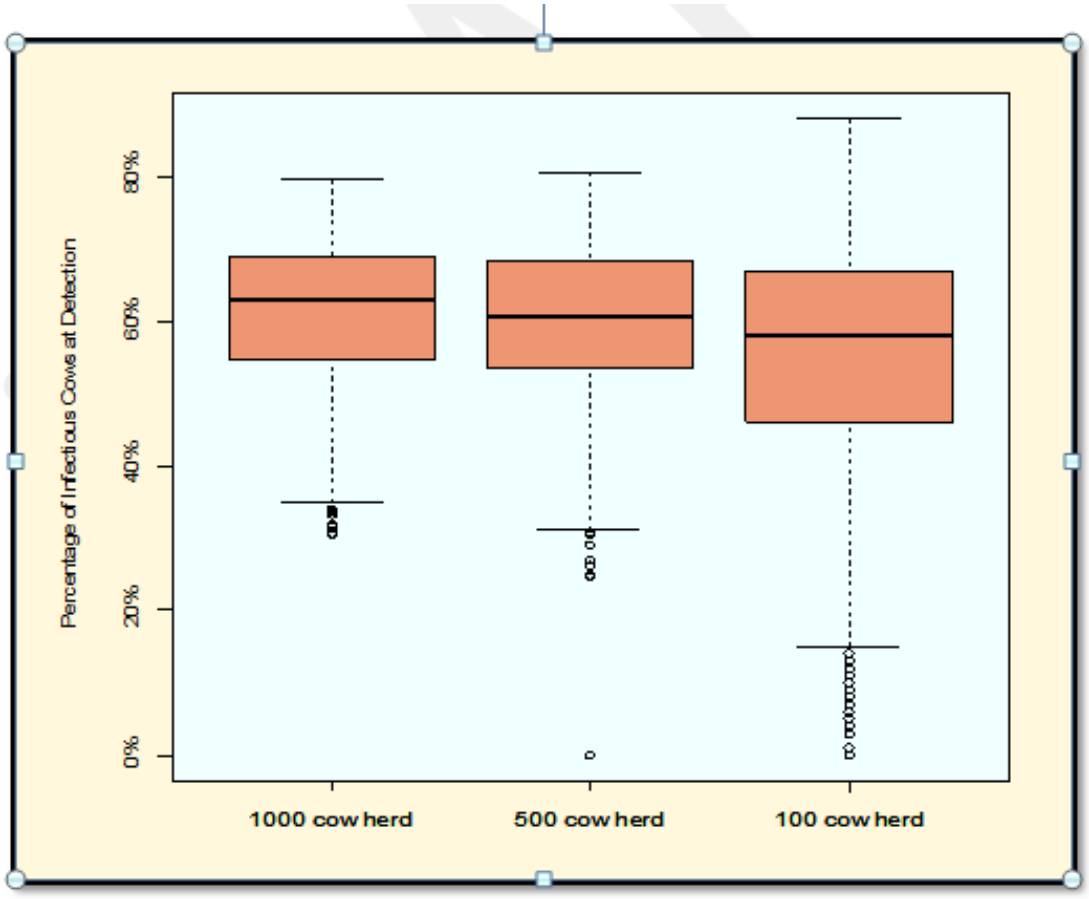

Figure 8. Percentage of infectious cows at time of detection (using 1 percent surveillance level) by herd size.

Table 9 summarizes key modeling results for the days to detect disease and the number of preclinical and clinical cows on the day of detection for the 3 dairy sizes using a surveillance level of 5 or 10 percent. This data is presented together, as it is assumed that disease is likely to be detected when the number of clinical cows is in this range. The results indicate that on average it will take 5 to 6 days to detect disease, regardless of the herd size and greater than 60 percent of the herd is infectious (preclinical and clinically) at this time. Comparison of the results for the 5 and 10 percent surveillance level for the 2 larger farm sizes indicates that the number of preclinically infectious cows plateaus on the day of detection, but the number of clinically infectious cows increases significantly from the 5 to 10 percent level due to preclinical cows transitioning into the clinical phase. These results are consistent with the relatively fast disease spread (based on a high adequate contact rate) and a long preclinical period.

Table 9. Estimates of key results, at the time of disease detection, using 5 and 10 percent surveillance levels.

Mean Values for 5 and 10% Surveillance Levels	Percent	100-cow Herd	500-cow Herd	1,000-cow Herd
Mean days to detect disease post infection	5%	4.8 (3-8)	5.2 (4-8)	5.4 (4-8)
	10%	5.2 (4-8)	5.7 (4-9)	5.9 (4-9)
Mean number of preclinically infectious cows	5%	63 (54-72)	320 (298-339)	640 (605-670)
	10%	63 (55-71)	318 (296-339)	634 (598-667)
Mean number of clinically infectious cows	5%	12 (5-21)	57 (27-95)	113 (54-190)
	10%	18 (10-28)	90 (53-134)	183 (106-265)

Table 10 presents the log mean FMD milk titer data using the 5 and 10 percent surveillance levels and for the combined data (combined and analyzed together) for these two surveillance levels. The log mean FMD milk titer increases significantly from the 5 to 10 percent surveillance levels due to the increase in cow numbers transitioning into both the preclinical and clinical phases. The combined data (5 and 10 percent combined) indicates that the mean titer will range from 3.36 to 3.55 log10 PFU/ml with a 5^{th} and 95 percentile values that range from 2.58 to 4.03 log10 PFU/ml for all farms sizes.

Table 10. Summary of estimated milk titers on the day of detection using 5 percent, 10 percent and combined 5 and 10 percent surveillance levels.

Log mean, 5th and 95th percentile FMD Titer in Bulk Tank PFU/ml)	100-cow Herd	500-cow Herd	1,000-cow Herd
5% surveillance level	3.26 (2.33-3.86)	3.43 (2.86-3.90)	3.44 (2.88-3.91)
10% surveillance level	3.47 (2.83-3.96)	3.59 (3.12-3.97)	3.60 (3.15-3.99)
5% and 10% data combined	3.36 (2.58-3.92)	3.51 (2.96-3.94)	3.55 (2.94-4.03)

The research studies used in this assessment report their data in units of PFU/ml. This data was converted to units of $TCID_{50}$/ml for comparison to well-known published benchmark values for FMD. **Table 11** used the combined data (5 and 10 percent levels) results for milk titer reported in **Table 9** and converts the values to units of TCID50/ml and then to an integer (non-log) value. The mean FMDv milk titer for all farm sizes ranged from 3,236 to 5,012 $TCID_{50}$/ml indicating that there is significant risk from inhalation exposure to less than 1 ml of infected milk. Cattle are very sensitive to inhalational exposure to virus, requiring as little as 10 $TCID_{50}$ to initiate infection (Alexandersen et al., 2003c). Comparison of the modeling results to studies that specifically evaluated aerosol exposure in cattle (**Table 12**) indicates that the estimated values are well within the range that can cause success infection in susceptible cattle via all 3-exposure methods.

The mean $TCID_{50}$ values presented in **Table 11** are 1 to 2 orders of magnitude greater than the lowest doses reported to cause disease. This indicates that as little as $^1/_{10}$ to $^1/_{100}$ of 1 ml of milk could pose a risk for exposure via inhalation. The routes for inhalation of virus would include activities such as splashing of milk, dry down of contaminated milk onto soils or feed that is subsequently aerosolized into cow areas, and cleaning or movement of environmental media that is contaminated with FMD contaminated milk and aerosolized. In contrast, the oral dose to cause infection in cattle is listed as 10^5 to 10^6 $TCID_{50}$.

Table 11. Mean milk titer in $TCID_{50}$/ml and conversion to non-log value.

FMD Milk in Bulk Tank	100-cow Herd	500-cow Herd	1,000-cow Herd
Log mean concentrations (5th and 95th percentiles) $log_{10}TCID_{50}$/ml*	3.51 (2.73-4.07)	3.66 (3.11-4.09)	3.70 (3.09-4.18)
Mean concentration (5th and 95th percentile) $TCID_{50}$/ml**	3,236 (537-11,749)	4,571 (1,288-12,303)	5,012 (1,230-15,136)

*conversion from log_{10} PFU/ml to log_{10} TCID50/ml value

**conversion from log value to integer value

Table 12. Viral titers that can cause infection by aerosol exposure.

Method of Exposure	Dose Range Causing Infection	Reference
Mask with artificial aerosols generated from spinning top apparatus	12.5 to 160,288 $TCID_{50}$	Donaldson et al., 1987
Mask exposure to natural pig aerosols	25 to 127 $TCID_{50}$	Donaldson et al., 1987
Indirect contact exposure to pigs aerosols	254 to 25,403 $TCID_{50}$	Donaldson and Kitching (1989)

8.5 Chapter Conclusion

The results of SLIR modeling indicate that disease will spread rapidly within dairy farms, with approximately 60 percent of the cows in an infectious stage, regardless of the farm size or surveillance level on the day of detection. The mean number of days to detect disease ranges from 3.9 to 5.9 days for all farm sizes and all surveillance levels. The disease will most likely be detected when 5 to 10 percent of the herd is showing clinical signs, which occurs day 5 to 6 post infection of the herd (mean value) with a range of 2 to 9 days (5^{th} and 95^{th} percentile values) for all farm sizes combined.

The number of preclinically infectious cows on the day of detection is similar for both the 5 and 10 percent surveillance level (respectively) as follows: 100-head herd (63, 63); 500-head herd (320, 318); and 1,000-head herd (640, 634). The mean number of clinically infectious cows varies more dramatically on the day of detection, due to the model design: 100-head herd (12, 18); 500-head herd (57, 90); and 1,000-head herd (113, 183) for the respective 5 and 10 percent surveillance levels. The number of clinically infectious cows reported at each model "check time" exceeds the surveillance level trigger of 5 and 10 percent (e.g., 50 and 100 cows for a 1,000-head herd) due to the rapid spread of disease within the last 24-hour period when the model last "checked" for clinical stage animals. The similarity in results for the 5 and 10 percent detection levels reflects the rapid transition of animals from the latent to preclinical to clinical stages, once disease is established within the herd.

The combined data for the 5 and 10 percent surveillance levels for each farm size indicates that the mean log milk titer (and 5^{th} and 95^{th} percentile values) will vary from 3.36 (2.58 to 3.92) to 3.51 (2.96 to 3.94) and 3.55 (2.94 to 4.03) log_{10} PFU/ml for the small to largest farm size respectively. There is significant variation in the milk titer during the initial 5 days post-infection. Around day 5 to 6 post-infection, the milk titer reaches a plateau at approximately the same time as the detection of disease.

The estimated concentrations of virus in milk at the time of detection were converted to $TCID_{50}$ values for comparison to published benchmark values, which have been shown to cause infection in cattle. The mean FMDv titers in milk from the model (and the 5^{th} and 95^{th} percentile values) for each farm size were 3,236 (537 to 11,749); 4,571 (1,288 to 12,303); and 5,012 (1,230 to

15,136) $TCID_{50}$/ml for the smallest to largest farms respectively. These values are orders of magnitude greater than the minimum infectious doses for cattle via inhalation exposure. Experimentally, the minimum infectious dose for a cow via mask exposure to naturally generated pig aerosols has been shown to be as low as 25 to 257 $TCID_{50}$. Susceptible cattle exposed to FMDv aerosols generated from indirect exposure to crated pigs for 60 minutes at concentrations of 254 to 25,403 $TCID_{50}$ FMD resulted in clinical disease. Cattle are most susceptible to inhalation exposure to FMDv and less susceptible to oral exposure (10^5 to 10^6 $TCID_{50}$) and nasal instillation (10^4 to 10^5 $TCID_{50}$) exposure.

The mean titers for FMDv in milk modeled in this report are one to two orders of magnitude greater than the lowest doses reported to cause disease via inhalation routes. This indicates that as little as $^1/_{10}$ to $^1/_{100}$ of 1 ml of milk could pose a risk for inhalation exposure. Cattle would not inhale milk directly, but could be exposed to contaminated milk via dairy activities that aerosolize milk and virus. The routes for inhalation of virus would include activities such as splashing of milk, dry down of contaminated milk onto soils or feed that is subsequently aerosolized into cow areas, cleaning activities in the milking parlor and alleyways, or movement and aerosolization of environmental media that contains FMDv contaminated milk.

9. Contaminated Milk Pathways

This section addresses the pathways that allow movement of virus from an infected but undetected dairy farm and the pathways that allow deposition or release of virus onto susceptible premises through the transport of raw milk. The site conceptual model (Chapter 7) presents the background information on routes for transport and movement of virus. Once FMDv enters susceptible premises—via the transport of raw milk and is deposited onto areas including the grounds, buildings, and equipment—there are numerous routes for movement of the virus into animal areas through routine dairy farm activities. These routes for movement on-farm are not specifically addressed in the risk assessment. It is assumed that if the virus is successfully transported and released on susceptible premises—at concentrations that pose a risk to livestock—there will be a high probability of the virus being moved into animal areas resulting in viral exposure and development of disease. The analysis of all pathways included a review of applicable literature, expert opinion from dairy industry and other scientific disciplines, personal observations by the risk analyst and informed professional judgment. The chapter is divided into the following pathways for analysis:

Likelihood of FMDv-infectious milk, that leaves an infected but undetected farm, results in contamination of the environment and the tanker and hauler/ at doses sufficient to cause disease in susceptible livestock:

- Likelihood of milk containing FMDv emanating from a milk tanker via bioaerosolization.
- Likelihood of contamination of the hauler and truck cab by FMDv contaminated milk spillage.
- The likelihood of external contamination of the tanker (includes storage compartment and transfer hose) through FMDv contaminated milk spillage.

Likelihood that a tanker, that does or does not undergo the CIP process, will contaminate an uninfected farm with residual virus at doses sufficient to cause disease in susceptible livestock:

- Likelihood that virus present in residual milk left in tankers, that do not undergo the CIP process, will result in contamination of an uninfected farm.
- Likelihood that virus present in residual milk, left in tankers that have undergone the CIP process, will result in contamination of an uninfected farm.

Likelihood of direct and indirect contamination of an uninfected farm, processing plant, or other premises by spillage of infectious milk during transport

- Likelihood of direct release of contaminated milk, via spillage from the transfer hose, on a susceptible farm.
- Likelihood of direct release (deposition) of contaminated milk, from external tanker surfaces (includes storage compartments and external surface of the transfer hose) and the hauler's clothing and boots, onto areas of a susceptible farm.
- Likelihood of indirect release (deposition) of contaminated milk—through accidental losses from the tanker or hauler resulting in cross-contamination of a person, vehicle, or farm—from roadway travel or other stops.

9.1 Direct Routes for Contamination of the Hauler and Tanker

Likelihood of FMDv contaminated milk that leaves an infected but undetected farm results in contamination of the environment and the hauler and tanker surfaces at concentrations sufficient to cause disease in susceptible livestock.

The main pathways for transportation of FMDv contaminated milk from an infected, but undetected, farm are:

- Contamination of the hauler's clothing, boots, and hands by spilled milk
- Milk spilled in tanker compartments that leaks out
- Residual milk left in the transfer hose and spilled onto the ground and floor of the milk house
- Milk contamination on the exterior surface of the transfer hoses and the tanker
- Milk accidentally lost during transport of milk on the road
- Potential generation of bioaerosols during pumping activities and from loss of milk during transport

9.1.1 Background: Tanker Characteristics

The basic design of a milk tanker is similar to that of a stainless steel insulated bottle. The tanker is double-walled and not refrigerated. Milk cooled to 36 to 37 °F (2 °C) is pumped from the bulk tank on a farm into the tanker. Milk is pumped from on farm bulk tanks through a transfer hose into the bottom of the tanker, so that splashing and movement of milk is expected to be minimized. At the plant yard or the first stop of the day, the interior of the tank and the gasket that is seated between the inner lid and the tanker wall are inspected for abnormalities by the hauler. During pumping, the latches that hold the dome lid cover and inner lid in a closed position may or may not be placed in a slightly opened (loosened) position, depending on State regulations. The PMO requires that the manhole is covered during pumping activities. There are two air vents systems– one or more vents are located in the inner lid (Runovent® or Olsen®

vent) and a smaller vent is present in the dome lid. Air displacement during pumping leaves the tanker primarily through the vent system or through upward lifting of the inner lid and the dome lid cover.

Personal observation by the risk analyst on Colorado dairies indicated that the majority of airflow exiting the tanker occurs around the edges of the lid system and not through the air filters. The two types of air vents used are the Runovent® and the Olsen® vent (DBL Tank, 2011), which function for pressure equalization during transport and pumping. The Runovent® plastic air filter has a corkscrew shape and is typically found on farm pickup tankers. This vent is more prone to loss of milk compared to the Olsen® vent, which has a spring-loaded pop-up design.

Figure 9. Tanker lid assembly showing manhole cover and inner lid *(courtesy of Danelle Bickett-Weddle, Iowa State University).*

Trucks may have metal spill dams that surround the manhole cover and contain tubes that are positioned down the side of the tanker, within the ladder rails or between the walls of the tanker to the undercarriage of the tanker. The tubes allow spilled milk to drip onto the ground beneath the truck. **Figure 9** shows the white gasket on the edge of the inner lid and two blue Runovent® filters positioned within the inner lid. The manhole cover is opened up to a vertical position and the four latches can be seen at the edges of the inner lid. **Figure 10** shows a tanker with a spill dam and the vent in the left side of the manhole cover. The person's hand is grasping the Runovent® air filter and the latch arms are visible in the photo.

Figure 10. Tanker lid assembly showing spill dam and manhole cover vent *(courtesy of Dr. Danelle Bickett Weddle, Iowa State University).*

9.1.2 Milk Spillage and Losses during Conveyance

Based on expert opinion, the amount of milk lost during conveyance is very small with average losses from tankers ranging from 2 cups to 1.5 gallons. There is also some loss of milk at each unhooking of the transfer hose with losses ranging from a few cups up to a gallon during uncoupling activities. Five to ten gallons of milk can be left in the truck mounted transfer hose after pumping. When the transfer hose is rolled up, milk may spill on the ground and within the storage compartment of the tanker prior to capping. For truck

mounted transfer hoses, the hose is always attached to the valve assembly and not disconnected after pumping. Milk may be pumped into the rear of the truck or into the underside (belly pump) in the middle of the tanker. The size of the transfer hose is generally 2.5 inches in diameter and 25 ft. long and can carry 1,250 lbs of milk/minute. Some hoses can carry up to 1,800 to 1,900 lbs/min (Gibson, 2011).

Milk can be forced up through the vents only under certain conditions. Depending on the fill level of the tanker, sudden stops or changes in direction by the tanker can create a large wave of milk movement within the tanker. For milk to leak out of a tanker during transport, it has to move up through the air vent, leak out under the dome lid cover and spill down the sides of the tanker (without spill dams) or funneled down through the vertical tubes onto the ground (trucks with spill dams). For a large amount of milk to escape the tanker, the latches would have to be violently loosened to allow the inner lid to open and allow a large surge of milk to exit the tanker, which could occur during an accident. According to industry experts, milk loss through the top of the tanker is generally minimal and occurs infrequently. Accidental loss of milk (due to failure to close the nuts and fitting tightly) could lead to loss of milk during transport— but is an uncommon occurrence. According to industry experts, most milk losses occur on the farm. At the receiving plant, it is not unusual to see small amounts of spilled milk under the dome lid or streaked down the sides on tankers that have travelled long distances between States (personal observation by Risk Analyst, 2011).

9.2 Bioaerosol Route

Likelihood of milk containing FMDv emanating from a tanker via bioaerosolization.

This section describes the factors that influence potential aerosolization of infectious raw milk through milk pumping activities on an infected but undetected source farm, and subsequent exposure on non-source farms or the processing plant. If aerosols are generated during the pumping of milk, and exit the tanker beneath the inner lid/dome lid or through the vent, livestock in close proximity may be exposed to infectious particles. The scenarios in which aerosolization of FMDv infected milk may occur are: (1) during pumping of raw milk into the tanker and passage beneath the inner lid or through the air vent, and (2) via loss of raw milk through the air vent during conveyance on the road. There has been no direct research on aerosolization of milk during pumping and transport activities in an outbreak. Recent review articles have focused on fomites—including the tanker, driver's clothing and boots, and the spillage of milk—as the most likely sources for the movement of infectious virus by milk tankers during outbreaks in the United Kingdom.

Spread of the virus by vehicles, and personnel moving between farms, is accepted in the FMD literature to be part of the epidemiology of the disease. The generation of bioaerosols out of the tanker vents, during milk pumping, has been considered a risk. Experimental investigations conducted at the Microbiological Research Establishment, Wiltshire, United Kingdom in 1968

showed that when *Bacillus globigii* spores were added to milk in a tanker, the number of spores recovered from the sampled vent air was a very small proportion of those present in the milk (Harper, 1968). *Bacillus globigii* is a gram-positive spore forming anaerobic bacteria that is used as a biological tracer. It was concluded that ground contamination was a more likely route of dissemination of FMDv than airborne spread. Although the probability of spread by infective aerosols from milk tankers may be very low, some veterinary authorities have included steps in their contingency plans to eliminate it (Donaldson, 1997).

9.2.1 Overview of Microbial Bioaerosol Science

The science of bioaerosols is extremely complex and requires an understanding of microbiology, biology, chemistry, meteorology, and aerosol physics (Mohr, 2005). The sections below focus on the key points for general bioaerosol behavior that may pertain to FMDv associated bioaerosols. Appendix F presents detailed information on general aerosol science on the sources, generation, and transport of bioaerosols.

Gilbert and Duchaine, 2009, provided a general overview of aerosol science in their paper *Bioaerosols in Industrial Environments: A Review*. Aerosols refer to an assortment of liquid or solid particles suspended in a gaseous medium (Gilbert and Duchaine, 2009). Bioaerosols are aerosols that contain microorganisms such as bacteria, fungi, and viruses—or organic compounds (endotoxins, metabolites, toxins, proteins from animals and plants, and other microbial fragments) (Macher et al., 1999). The ability to generate bioaerosols depends on the source, aerosolization mechanisms, environmental conditions, and composition (Pillai and Ricke, 2002). Bioaerosols vary in size from 20 nm to >100 µm in diameter. Almost any environmental reservoir for microorganisms, such as fresh and marine surface waters, soil, plants, wastes, and animals is susceptible to being a source of bioaerosols. Bioaerosols generated from water sources are generally surrounded by a thin layer of liquid that rapidly evaporates to give droplet nuclei. Droplet nuclei are the dried residue of larger aerosols that can remain airborne indefinitely on air currents.

Transport of bioaerosols, and survival of airborne microorganisms, are influenced by many physical and environmental factors. The size, shape, and density of bioaerosols are of particular significance to transport because they are related to the aerodynamic diameter, which controls the settling velocity (Cox, 1989; Mohr, 2007). Bioaerosols between 1 and 5 µm normally follow the streamlines of surrounding air, making them less susceptible than larger particles to impact surfaces and deposition (Mohr, 2007).

Temperature, relative humidity (RH), and air currents also affect the transport and generation of bioaerosols. Relative humidity, temperature, oxygen concentration and solar irradiance are among the factors influencing the fate and survival of airborne microorganisms (Mohr, 2007). A decrease in RH causes the water surrounding bioaerosols to evaporate, forming droplet nuclei aerosols. A lack of water can result in the inactivation of many microorganisms. The effect of temperature on bioaerosols' stability is difficult to assess as the vapor pressure and the RH of the

system depends on the temperature. Vulnerability of airborne microorganisms to environmental stresses also depends on the type of organism and the state in which it is found in the air.

9.2.2 FMD Windborne Spread Knowledge

The climatic conditions that are required for animal source aerosol spread of FMD are presented in Section 5.2.3of the Hazard Identification. It is unknown if milk bioaerosols would require the same type of environmental conditions to remain viable and potentially lead to infection of susceptible livestock.

9.2.3 Expert Opinion on Bioaerosolization

We queried seven bioaerosol experts on the potential for generation of bioaerosols during pumping and transport, and the expected behavior of the aerosols. The experts have different professional backgrounds and expertise within the field of aerosol science, including research in microbial aerosols, industrial hygiene workplace exposure, and bioterrorism aerosol behavior. The last experts to be interviewed had experience with bioterrorism aerosol research, which is probably the most applicable research for understanding milk bioaerosols. The list of experts interviewed, the questions asked and a summary table of their answers are presented in **Appendix E**. Responses were compiled and compared for consensus between experts. The explanation of aerosol behavior varied slightly between experts, but the overall conclusions of the probability of occurrence of bioaerosols did not vary significantly.

There are no studies on milk bioaerosols or the physical chemistry of milk within a tanker available in the literature. Because aerosol science is a very complex field, aerosol characteristics and behavior is measured quantitatively using sophisticated sampling methods and equipment. Questions on aerosol behavior cannot be accurately modeled mathematically, as it requires knowledge of the concentration of aerosols, the size distribution of aerosols, the media composition, and the environmental/atmospheric conditions under which they are generated. Due to the lack of information on milk aerosols and chemical/physical properties of milk within a tanker, the experts' opinions and rationale were based on extrapolation of their knowledge in aerosol science. All of the experts agreed that experimentation is required to accurately answer these questions and to validate their opinions.

9.2.4 Expert Opinion Summary

All of the experts agreed that during pumping of milk at the first farm, transport, and pumping at subsequent farms, there is a low or very low probability for aerosol generation and escape of bioaerosols from the tanker in concentrations that pose a risk to livestock. However, the probability of aerosols being generated cannot be discounted, as bubble bursting, milk movement and energy input into the tanker through pumping could produce a large number of aerosols. The question is whether the population of aerosols will contain an adequate concentration of small particles with infectious virus within them to pose a risk to susceptible livestock. The suspected

behavior of bioaerosols is presented below which represents a consensus of the majority of expert's opinions.

- Aerosols would need to be in the size range of 5 to 10 µm in order to escape the tanker in the airstream during pumping activities. Most particles/droplets generated within the tanker will be large particles and impact the tanker walls, vent, inner lid, and dome-lid (dust cover) and fall back into the fluid milk. It takes a lot of energy input into a system to produce small particles less than 10 µm in size. For aerosols generated from liquids, a distribution of sizes is produced. Ninety percent of the mass of the bioaerosols generated is accounted for by larger particles. Within the aerosol population generated, there can be a large population of small particles (less than 10 µm), but they account for a very small percentage of the total mass. Six of seven experts made similar statements.
- Five of the seven experts believed that there would be enough energy from sloshing and bubble bursting activity to produce aerosols during the initial 10 minutes or more of pumping. The most important question is what is the percentage of aerosols that exit the tanker and remain viable? Some percentage of the aerosols will impact the tanker walls, vent, and dome lid assembly and some will escape. The concentration and size distribution of the particles that could escape the tanker are unknown.
- Three factors are working in favor of not generating aerosols in the threat range of 1 to 10 µm: (1) the temperature of the milk <40 °F; (2) the relative humidity of the headspace; and (3) the percent solids found in milk (approximately 13 percent). It is likely that aerosols will be produced within the tanker due to bubble bursting activity during the initial stages of pumping. Because of the high humidity levels in the tanker, larger droplets will be produced that will rapidly settle back into the fluid level. Ninety percent of the material will fall back into the fluid milk.
- Research with liquids aerosolized within the Ambient Breeze Tunnel (wind tunnel) at U.S. Army Dugway Proving Ground showed that the aerosols do not dry down fast enough to be carried down the tunnel when the temperature of the liquid is below 40 °F. Assuming the headspace of the tanker is also the same temperature as the milk, they expect that any aerosols generated during transport will fall back into the liquid prior to pumping at the next farm. Once the RH goes above 50 percent, the properties of aerosols change. More particles will agglomerate and fall back into the liquid. High humidity will cause rapid settling of aerosols/particles.
- Based on the percent solids in milk, and assuming a 50 µm droplet size, one can reasonably expect a ~10 µm dry aerosol to be produced. It is expected that greater than 50 percent of the dry aerosols will be large and will fall out, even if they escaped the tanker dome lid assembly. Sloshing of milk during transport could produce large numbers and variable sizes of aerosols. The diameter of the aerosol is the critical point in

determining settling velocity. As the size of particles increase, they will settle exponentially faster.

- Pumping horizontally into the tanker should minimize splashing and any "fountain effect" which could produce bioaerosols. The worst possible time to produce aerosols is when the milk is just starting to be fed into the tanker. Initial pumping activities are more likely to produce large particles (≤100 µm in size), but some small particles could be generated. The small particles would represent a small percentage of the overall population of particles generated. Although small particle generation is unlikely, it cannot be ruled out. Once milk is pumped in under a liquid layer, there is not a concern for generation of bioaerosols (5/7 experts agreed).
- Regarding pumping at a subsequent farm – if the pumping of milk into the tanker is under the fluid milk level, it is not a concern, because of the lack of bubble bursting activity and the lack of energy input into the system. If aerosols were present in the headspace upon entering a subsequent farm, it could present a risk for exiting the tanker during pumping, if enough particles were less than 10 µm in size.
- The Runovent® will inhibit the majority of particles from exiting the tanker due to the torturous path it creates for air movement during transport. It is designed as an impaction device. If the net air movement is not through the Runovent®, but is by the air lifting the inner lid of the tanker and exiting via this route, there will be a greater chance for particles to remain in the airstream. It may be a combination of flows that need to be characterized.
- Transport of milk may or may not produce aerosols from movement of the milk in the tanker. The droplets and aerosols produced would be large particles and settle back into the liquid quickly. Two experts thought there could be a small aerosol population within the headspace of the tanker that could potentially exit the tanker during pumping at a subsequent farm.
- Regarding accidental loss of milk during transport, most of the experts felt that if the milk adheres to the outside walls of the tanker (as suggested by the interviewer), and the milk dribbles out from underneath the dome lid and is not shot out under pressure, there is little opportunity for aerosol generation. Wind shear could produce aerosols, but most experts were skeptical about producing small size particles that would be viable. Pneumatic sheering can produce an aerosol, but it generally has the effect of killing most of the viable material. If milk dribbled onto the road from trucks with spill dams, the milk could be re-suspended by another vehicle's tires and/or subject to road shear.
- Milk may stabilize the bioaerosols, as skim milk has been shown to be a stabilizer for other bioaerosols, therefore milk (or its components) may protect the virus. The survivability of the milk bioaerosols is unknown.

- Particles of 5 µm or less act as vapors and can move in and follow an airstream without impacting obstacles. This is the size of particle that represents the worst risk.
- If the tanker is stationary for a short time span prior to pumping (approximately 10 minutes or more), the settling velocity of aerosols will cause most particles to settle out into the fluid. Ninety degree turns in a vent or dome lid assembly will remove particles 1 to 10 µm in size through impaction. This knowledge could be used to design the vent and modify the dome lid assembly (5/7 experts agreed).
- There are multiple ways to design and experimentally measure the air and aerosols that could exit the tanker. Dr. Peter Raynor stated the surrounding air can be measured (sampled) with a sampling instrument to observe if anything is coming out of the tanker. It is possible to get a sense of milk-based aerosols without looking specifically for viruses or bacteria. Experiments would answer many questions better than reliance solely on expert opinion.

Likelihood Estimate: The overall likelihood of bioaerosols emanating from a tanker and spreading infectious virus through milk collection and transport activities is estimated as low to very low.

This estimate was based on review of expert opinions, observation of milk tanker design and operations, and current knowledge of FMDv epidemiology. All of the experts agreed that the ability of bioaerosols to come into contact with susceptible livestock depends on proximity, temperature, and wind speed. This likelihood estimate, and the uncertainty surrounding it, may be revised as further information or experimental data become available. This estimate does not include the risks associated with the truck, hauler, equipment, or fomites as a source of disease spread.

9.3 Spilled Milk Route

Likelihood of contamination of the hauler and tanker cab by infectious spilled milk.

Contamination of the hauler will occur through contact with spilled milk during milk sampling and pumping activities. Milk is routinely spilled on the floor during pumping activities. Connection of the transfer hose to the bulk tank can result in the hauler having direct contact with spilled milk on the floor and the outside of the milk house during disconnection of the hose. Spilled milk and fomites can cause external contamination of the transfer hose. For tanker mounted transfer hoses, capping and rolling up of the hose and placement into the storage compartment can lead to milk spillage in the compartment and onto the ground that can contaminate the hauler. The driver's clothing, hands, and boots will potentially have contact with all of the surfaces where spillage occurs. Contamination of the hauler will be carried into the cab.

Likelihood Estimate: Likelihood of hauler and tanker cab contamination by spilled milk: moderate to high.

Likelihood of external contamination of the tanker (including storage compartment and transfer hose) through milk spillage

Residual milk that is left in the transfer hose can be spilled from the hose during placement into the storage compartment after pumping activities are finished. Leakage of milk within the storage compartment can lead to external contamination of the tanker tires and undercarriage from spilled milk leaked out of the compartment.

Likelihood Estimate: Likelihood of external tanker, storage compartment, and transfer hose being contaminated through milk spillage: moderate to high.

9.4 Clean-in-Place Route

Likelihood that a tanker that undergoes, or does not undergo, the CIP process will contaminate an uninfected farm with residual virus at doses sufficient to cause disease in susceptible livestock.

9.4.1 Clean-in-Place (CIP) Evaluation

This section addresses the risk of a tanker going to a non-infected premises following unloading of raw milk from an infected but undetected farm, resulting in entry of infectious virus onto a susceptible farm through residual milk left within the tanker. Within a 24-hour period, tankers may collect milk from several farms – and travel to the processing plant multiple times – to unload milk prior to performance of CIP. After unloading, the only pathway for milk to leave the tanker is through residual milk that is left in the transfer hose and spilled milk within tanker compartments, which could leak onto the ground or floor at the next farm. Milk left within the tanker, after unloading, cannot exit the tanker unless it can flow through the truck mounted pump due to a malfunction, or the bolts and cap on the entry port not correctly tightened (tankers without truck mounted pumps/transfer hoses).

Under normal conditions, the unloading of milk at the milk plant will remove all of the milk within the tanker. Milk is pumped into the plant silos using the processing plant's equipment. Very little residual milk is left in the bottom of the tanker after unloading (K. Johnson, personal observation, 2012-2013). Small milk-soils complexes (discussed below) may form on the walls of the tankers, which are foamy in appearance and are more likely to be present in tankers that have travelled for long distances (D. Davis personal communication, February, 2012). A film of milk could be present on the walls of the tanker – but if it occurs, is not readily observable (K. Johnson, personal observation, 2012-2013). During unloading of milk, the inner lid/dome lid assembly is left open after collection of the quality control samples. The hauler retires to a waiting room, or remains in their truck, until the process is complete.

According to the current PMO regulations, the internal tank—and associated equipment—is required to be cleaned every 24 hours. The bulk-milk hauler is responsible for assuring that the

milk tanker has been properly cleaned and sanitized at a permitted facility. During CIP, the gasket associated with the inner lid is removed and washed, the transfer hose is cleaned, the pump is disassembled and cleaned, and the storage compartment is washed. There are no specific time requirements for the external washing of tankers; this is left to the discretion of the company. The PMO regulations pertaining to the cleaning and sanitizing of milk tankers and equipment are presented in Appendix A.

9.4.2 Cleaning and Disinfection of Dairy Equipment

The article *Practical Hygiene and Disinfection on Dairy Farms* (Sigurdson et al., 2004) provides a useful overview of the cleaning and disinfection information applicable to CIP systems used for both on-farm bulk tanks and milk tankers after the unloading of milk. The following background information on cleaning and disinfection and CIP was obtained from this document. Soil on dairy equipment consists of any material that is undesirable for producing safe, quality, and wholesome milk products. Invisible soils include microorganisms, while visible soils on equipment surfaces can be dirt, deposits, residues, or films, all of which must be removed. Anything that contacts equipment surfaces causes soiling, which can result in soil complexes, which can vary in composition. Predominant soil complexes on dairy equipment are the residues from milk and water, but can also include soils from personnel and other sources.

Milk is composed primarily of water (approximately 86 percent), but also contains protein (3.4 percent), fat (3.7 percent), sugar as lactose (4.9 percent) and minerals (0.7 percent). The compounds in milk are susceptible to different cleaning agents as follows:

- Milk soil, which is found on equipment, is comprised of fats and proteins, and can be removed with alkaline detergents
- Minerals are solubilized with acid detergents
- Lactose (milk sugar) is a carbohydrate soil that is easily removed with warm water.
- Proteins are one of the more difficult soils to clean from equipment. The removal of protein soils depends on several factors including the nature of the protein, degree of denaturation of protein (physical change due to heating), and the condition under which the protein is deposited.

Fresh unheated milk is easily removed by rinsing. Milking machines and all milking equipment and utensils on the farm must be thoroughly cleaned and sanitized after each milking. Milk can generate protein films that may be difficult to remove if allowed to dry and persist. Milk fat films usually appear as a greasy film on the surface. With proper rinsing, as much as 90 percent of the milk soil is removed. The remaining 10 percent soil load can be removed with sound CIP procedures.

If the milk soil is allowed to dry on the surface, a more, aggressive cleaning treatment is required. Soils, in combination with minerals, are referred to as “stone.” Milk stone is one of the

more complex soil deposits on milking equipment. Usually an alkaline wash, followed by an acid rinse, will remove milk stone (Sigurdson et al., 2004).

9.4.2.1 Four-by-Four Formula

There are four quality steps and four inter-related variables that are considered in the cleaning and disinfection of dairy equipment, which are referred to as the 4x4 formula. Cleaning consists of three distinct steps: pre-rinsing, washing, and post-rinsing. The fourth step is sanitizing and/or disinfecting, depending on the product and Environmental Protection Agency (EPA) product claim. The four-by-four cleaning steps include:

1. The pre-rinse step removes a large portion of the milk soil
2. Washing removes any remaining visible soils
3. The post-rinse cleans away the detergent and soils
4. Sanitizing and/or disinfection kill microorganisms that may cause spoilage, animal disease, or foodborne disease in everyday milk production practices

The four interrelated variables that impact the effectiveness of cleaning and disinfection are listed below. They can be adjusted to specific needs or cleaning applications.

1. Concentration of the cleaning agent
2. Water temperature
3. Time required for the cleaning cycle
4. Amount of mechanical or manual force required

Cleaners can be either alkaline detergents or acid detergents. The cleaning process, which is the application of the 4x4 formula, does not necessarily remove or kill microorganisms. A sanitizer is a substance that reduces the microbial contamination on inanimate surfaces to levels that are considered safe from a public health standpoint. The most common sanitizers used in the dairy industry are chlorine, iodophors, carboxylic-acid sanitizers, and acid sanitizers. Peroxy acids, also known as peracetic acid sanitizers, are the newest type of sanitizing compounds. Quaternary ammonium compounds are commonly used sanitizer, but to a lesser degree in the dairy industry, as even a small quantity in milk can inhibit the activity of starter cultures.

The protocol for CIP at the processing plant is similar to the CIP process for the on-farm bulk tank. This analysis will focus on the processing plant CIP process.

9.4.2.2 Generic CIP Process

The generic CIP process for on-farm bulk and milk tankers includes the following steps:

1. Pre-rinse with fresh water
2. Chlorinated (caustic) wash

3. Post-rinse with fresh water
4. Acid rinse
5. Fresh water rinse
6. Sanitizing wash

Steps 4 and 6 can be combined into an acid sanitizer for one step

Generic Processing Plant CIP Process

1. Pre-rinse with fresh water
2. Chlorinated (alkaline) wash to remove organic material. Chlorinated alkaline wash (4,000 to 5,000 ppm, 0.4 to 0.5 percent) of sodium hydroxide (NaOH) or potassium hydroxide (KOH) with sodium hypochlorite (NAOCl) (150 ppm). The temperature is 140 to 150 ºF (60 to 65 °C) for 20 minutes with a pH value of 11 to 12.
3. Post-rinse with water at room temperature (62 to 64 °F; 16.6 °C). *This step is optional.*
4. Acid rinse. The acid rinse step is designed to remove minerals and decrease the pH in the tank to below 4. Product examples include nitric phosphoric blended acid with a pH at or below 3. The typical wash circulates for 2 to 4 minutes at a temperature range of 125 to 135 °F (51.6 to 57.2 °C), although no temperature requirement has to be met. The primary purpose is to reduce the pH below 4 to mitigate mineral deposits and create an on-going effect of the chlorinated alkaline products on equipment.
5. Sanitizing. Sanitizers must be EPA approved products. There are three categories of sanitizers typically used on dairy farms:

Fatty acid-based sanitizers (acid sanitizer) with a working pH of 3

Peroxyacetic acid with a working pH of 4.5 to 5

Chlorine (100 to 200 ppm) with a working pH of 7 to 9

Only the fatty acid based sanitizers have sufficient acidity to act as the acid rinse also. Some dairy plants use enzymatic products, which replace the acid rinse step so there is an enzymatic wash and a sanitizer step. Water hardness determines if a rinse is used; hard water areas generally substitute the rinse with an acid sanitizer.

9.4.3 Likelihood of CIP Route of Contamination

Likelihood that virus present in residual milk left in tankers that do not undergo the CIP process will result in contamination of an uninfected farm.

After unloading of milk, only a small amount of milk and dried milk residue will be left in the tanker. Residual milk within the tank cannot exit the tanker unless there is a pump failure or inadequate closure of fittings on the pumping port. Krug et al. (2011) used higher titer virus stocks (minimum concentration of 4.8 $\log_{10}$ $TCID_{50}$) diluted in phosphate buffered saline PBS and dried on stainless steel and plastic surfaces to evaluate inactivation of FMDv (Krug et al., 2011). The initial drying process resulted in approximately a 2-log reduction in infectivity of FMDv on steel or plastic surfaces, thus the drying process itself will inactivate some of the virus. The lack of conducting the CIP process will not affect infectious milk left within the transfer hose and storage compartments. The external contamination of the tanker also remains unchanged.

Likelihood Estimate: Likelihood that virus present in milk residues left within the tanker that has not received CIP will result in release of virus on an uninfected farm is negligible to low.

Likelihood that virus present in residual milk left in tankers that have undergone the CIP process will result in contamination of an uninfected farm.

During the CIP process, the internal tank of the tanker, transfer hose, pump, and storage compartments are cleaned using high temperatures and chemicals with extreme pH. Research on FMDv has shown that the virus is inactivated by high temperatures (>50 °C) and pH levels of <4 or >11. Simulation studies of high-temperature, short-time (HTST) pasteurization at 72 °C for 15 seconds produce a 4-log reduction in viral titer. Most pasteurization inactivation studies involve heat treatment for periods less than 0.5 minutes—or use weak acids. De Leeuw et al. (1980) reported a 4-log reduction for FMDv in primarily infected milk at 60 °C within 1 minute (de Leeuw et al., 1980). Tomasula et al., (2007) reported up to a 4-log reduction in simulated HTST pasteurization for primarily infected milk treated at 72 °C or 95 °C for 18.6 or 36 seconds (Tomasula et al., 2007) . Chemical inactivation with weak acids, 0.5 percent and 1.0 percent citric acid, resulted in a 5-log reduction for FMDv dried on nonporous surfaces with a 10-minute contact time (Krug et al., 2011). Sodium hypochlorite (1,000 ppm) completely disinfected FMDv on stainless steel and plastic surface by 4 minutes. The CIP process uses time, temperature, and pH values that well exceed all of these values. Based on the titer of virus that will be found in milk $\log_{10}$ 2.64 to 3.60 PFU/ml, the process should inactivate the virus. The external contamination of the tanker is unchanged by this process.

Expert opinion was elicited from Dr. Peter Krug (ARS Plum Island, FMDv disinfection expert) on the efficacy of inactivating FMDv by the CIP process. He stated “the CIP process is very comprehensive. FMDv has little to no chance of surviving under those conditions – that first step at 60 °C alone will do most of the job. The acid rinse should finish the job. The 5 minutes with 200 ppm of chlorine probably is not as effective as the acid-based sanitizers, but by then I would expect FMDv would not have survived to that point anyway. However, that step would be useful

for acid-resistant viruses that somehow had survived the initial processes. Overall, the CIP process is impressive" (Krug, 2012).

Likelihood Estimate: The likelihood that viable virus will remain in the tanker after undergoing the CIP process and result in release of virus on an uninfected farm is negligible.

9.5 Direct and Indirect Routes for External Contamination

Likelihood of direct contamination of an uninfected farm and indirect contamination via processing plant, or other premises by spillage of infectious milk during the transport of raw milk.

9.5.1 External Tanker Surfaces (includes Storage Compartment and Transfer hose) and Hauler's Clothing, Boots, or Hands Route

Likelihood of direct release of infectious milk via spillage from the transfer hose on a susceptible premises and Likelihood of direct release of infectious milk from external tanker surfaces and the hauler's clothing and boots onto areas of a susceptible farm

When a tanker enters a non-infected farm, after collecting milk from an infected but undetected farm, the main routes for infectious milk to be released onto the susceptible farm are:

1. loss of residual milk from the transfer hose,
2. transfer of external contamination of the transfer hose to floors and parking area,
3. leakage of milk spilled within storage compartments onto grounds and
4. transfer of infectious fomites from the hauler's clothing, boots, hands, and equipment to milk house surfaces.

Residual milk left within the transfer hose can leak out during connection to the bulk tank and cause contamination of the ground around the entrance and floor of the milk house. External contamination of the transfer hose will also contaminate these areas. Spilt milk within storage compartments can leak onto the ground around the tanker. Contamination on the hauler's clothing, boots, hands, and equipment will result in fomite transfer to milk house surfaces, floors, and equipment.

Likelihood Estimate: Likelihood of spillage (direct release) of infectious milk from a transfer hose and external contamination of the tanker and hauler on a susceptible farm **is moderate to high.**

Likelihood of Indirect Release of contaminated milk through accidental losses from the tanker or hauler resulting in cross-contamination of another person, vehicle, or farm from roadway travel or other stops.

During transport of milk, accidental losses are uncommon through the vents or port system. If accidental losses occurred, it could lead to milk spillage onto the road or contamination of the exterior walls and tires of the tanker. Milk spilled onto roadways could be picked up by other vehicle tires or resuspended by tires into the air. Leakage of milk from the transfer hose within the storage compartment, or milk residues on the tanker tires, could occur, but the volume of milk lost to the ground/roadway would be expected to be very low. Contamination of other vehicle tires and undercarriage can lead to transport of virus onto farms. In the 2007 United Kingdom Surrey outbreak, mud contaminated truck tires were implicated as the source of virus on trucks that drove on a road adjacent to the farm, but not through the farm gate. (Ellis-Iversen et al., 2011). Any route that leads to spillage of milk can result in generation of fomites that may be transported onto a farm via multiple routes.

Likelihood Estimate: Likelihood of accidental milk spillage resulting in cross-contamination of a person, vehicle, or farm from roadway travel or other stop of the tanker leading is **low.**

9.6 Chapter Conclusion

The overall likelihood estimate for the generation and release of FMDv infectious milk bioaerosols exposing and causing disease in susceptible livestock from pumping activities on-farm, transport to another farm/processing plant, or from accidental loss of milk during transport is estimated as *low to very low* based on expert opinion, review of aerosol science, and observations of the analyst. No experimental data were found in the published literature on milk bioaerosols, or behavior of FMDv in aerosolized milk. It cannot be categorized as negligible, as there are no studies or data to support this likelihood level. The low milk temperature, high RH of the tanker's internal environment, and the high milk solids content are all factors that are unfavorable for generation of aerosols in the threat range of 1 to 10 µm. Based on liquid aerosol work at U.S. Army Dugway Proving Ground Life Sciences Division, "unless FMDv has an infectivity level down to a single virus, there is probably not an aerosol issue with milk tankers".

Accidental loss of milk during transport does not pose a risk for generation of bioaerosols as wind shear will cause desiccation of the milk very quickly. Once the milk bioaerosols are outside of the tanker and evaporate down to a solid particle, the viability of the virus is not known. As for pumping milk at a subsequent farm, only one expert felt there could be a risk of bioaerosols being pushed out through the vent system. This could occur if small bioaerosols were generated during transport in the headspace of the tanker and did not settle back down into the liquid layer within a short time. Most of the experts believed that large droplets could be generated during transport, which would then quickly settle into the fluid layer upon arrival at a second farm.

The likelihood of FMDv infectious milk leaving an infected farm through contamination of the hauler, cab, transfer hose, and exterior surfaces of the tanker (includes storage compartment and transfer hose) by spilled milk is moderate to high. These contaminated sources (from milk spillage) can serve to transfer virus to uninfected farms where virus can be transferred to many areas including the grounds around the tanker, milk house entrance, and milk house floor and

surfaces. The likelihood of direct contamination of the susceptible farm is rated as *moderate to high.* The CIP process will inactivate virus within the tanker, transfer hose, and storage compartments. If CIP is not performed, the transfer hose and storage compartments can still serve as sources for leakage of infectious milk. External contamination of the tanker remains unchanged, as the tanker is not washed after unloading milk or CIP. Accidental loss of milk during transport is unlikely, but leakage from storage compartments or loss of material (environmental media or milk) from tires could potentially contaminate roadways and be picked up by other vehicles travelling to farms. This route of exposure is rated a *low risk.*

10. Contaminated Environmental Media Pathways

This section analyzes the pathways for movement of FMDv contaminated environmental media (manure, urine, and other secretions mixed with soil) from an infected but undetected farm through the transport of raw milk which results in contamination of an uninfected premises with a virus at doses sufficient to cause disease in susceptible livestock. The overall risk from both the transport of FMDv-environmental contamination and infectious milk is presented at the end of the chapter. The chapter is organized similarly to the infectious milk pathway chapter and is divided into the following pathways.

Likelihood of FMDv contaminated environmental media that leaves an infected but undetected farm, results in contamination of the environment, tanker, and hauler at doses sufficient to cause disease in susceptible livestock.

- Likelihood of an individual hauler and tanker cab being contaminated by environmental media
- Likelihood of the external tanker surfaces (includes storage compartment and transfer hose) being contaminated by environmental media

Likelihood of environmental contamination leading to direct and indirect contamination of an uninfected farm.

- Likelihood of contaminated environmental media being deposited on an uninfected farm by the tanker and hauler directly
- Likelihood of indirect cross-contamination of people, vehicles, or farms via deposition of environmental media from roadway travel or other stop

Overall risk that transport of environmental contamination, and spilled infectious milk to other premises, will result in exposure of susceptible livestock on an uninfected farm.

- Likelihood that virus transported to a susceptible farm through contaminated environmental media and infectious milk may result in exposure of susceptible livestock.

10.1 Background on Environmental Contamination

This section of the risk assessment describes the likelihood that contamination of the tanker, hauler, and transfer hose by infectious environmental media—on an infected but undetected farm—will result in transfer of virus to susceptible farms and exposure of livestock. The term environmental contamination is used to describe the mixture of infectious manure with urine, salivary secretions, and milk (spilled from activities and leakage from cows) that can be mixed with soils from daily farm activities. The primary contributors to environmental contamination,

in latent and preclinically infected animals, are expected to be manure and urine—with smaller amounts of saliva and leaked milk. In this assessment, all contaminated environmental media is considered as a single source and is not evaluated separately. Other sources of environmental contamination are not addressed directly in this analysis.

Sellers and Parker (1969) reported that from the second to fifth day of infection, the environment can be contaminated by significant amounts of virus in feces (Sellers and Parker, 1969). There is limited data on virus titers in manure during different disease stages in the literature. No data could be found on titers of virus in environmental media. **Table B-6** provides an overview of **c**oncentrations of FMDv in manure that have been observed from experimental studies (range of 2.2 $\log_{10}$ $TCID_{50}$/g to 4.1 $\log_{10}$ $TCID_{50}$/g). The range of FMDv concentrations, noted in body secretions, is presented as follows (Alexandersen et al., 2003c; Honhold, 2011):

- Saliva: 10^{6} to $10^{8.8}$ $TCID_{50}$/ml
- Urine $10^{2.5}$ to $10^{5.5}$ $TCID_{50}$/ml
- Feces 10^{2} to $10^{3.3}$ $TCID_{50}$/ml
- Milk 10^{3} to $10^{4.5}$ $TCID_{50}$/ml

The approach for this evaluation was to estimate the potential volume of infectious manure that would be produced on an infected but undetected farm. Using the results of the SLIR model for the 1 percent surveillance level, we calculated the amount of infectious manure that could be produced before, and on the day of, disease detection. **Table 13** provides the parameters used in this calculation. The mean number of infectious cows (on day of detection, 1 percent surveillance level) was used with each cow producing 15 to 45 kg of manure/day. For a 1,000-head dairy, using the 616 (preclinical and clinical) infectious cows in the herd, the total quantity of manure produced ranges from 9,240 to 27,720 kg/day (20,370 to 61,112 lbs/day). This quantity of infected manure could be produced for 2 to 3 days prior to detection of disease. Thus, the sheer volume of infectious manure produced presents a risk for significant environmental contamination throughout the farm. In comparison, if disease is not detected until 5 or 10 percent of the herd is showing clinical signs, there are 753 or 817 infectious cows (mean number) in the herd at the time of detection, which represents a range of 24,901 to 81,052 lbs of contaminated manure produced daily.

On the day of detection, the mean FMDv titer in the bulk-tank milk was calculated to be 3.14 (2.33 to 3.75; 5^{th} and 95 percent percentile values) $\log_{10}$ PFU/ml which is equal to 3.29 (2.48 to 3.90; 5^{th} and 95^{th} percentile values) $\log_{10}$ $TCID_{50}$/ml. The concentration in feces and urine is generally less than milk, as noted above. Using a conservative approach, if the viral titer in manure is assumed to be one log lower than the mean milk titer, the resulting value of 2 $\log_{10}$ PFU/g (2.15 $TCID_{50}$/g)in manure, represents a $10^{6.7}$ theoretical total viral content (see Table B-6). The infectious manure would be mixed with manure from uninfected cows at about a 2:1 ratio. It requires a 10:1 ratio to decrease the concentration of FMDv by 1 log, thus dilution from

non-infected cows will not markedly lower the virus titer in manure. The mean value of 2.15 $\log_{10}$ TCID50/g (equal to 141 $TCID_{50}$/g) is well within the range of minimum infectious doses that cause disease via inhalation 12-254 $TCID_{50}$ (**Table 12**). This comparison also does not take into account the additional contributions by other secretions such as urine, saliva, respiratory secretions, and leaked milk, which would be expected to increase the viral titer. The amount of increase in viral titer is not known.

Table 13. Variables used to estimate the FMD virus titer in manure slurry and raw milk, from an infected undetected dairy herd (1,000-cow head).

Variable	Value	Source
The quantity of feces produced by a dairy cow on 1 day	15–45 kg	(Sellers, 1971)
Number of infected cattle excreting virus on day of detection (1% surveillance level) (Mean and 5th and 95th percentile)	616 (494–720)	Output from the disease transmission model
Quantity of feces produced by infected cattle per day	9,240–27,720 kg (20,370–61,112 lbs)	Based on 616 (mean number) of infectious cows
Quantity of feces produced by remaining 384 uninfected cows per day	5,760–17,280 kg (12,698–38,095 lbs) manure	Based on 384 susceptible cows (mean number) in the herd

Although the quantity of FMDv contaminated manure on the farm is substantial, the extent of contamination of surfaces outside the cattle pens is dependent on the frequency of movement of contaminated workers or equipment from pens to other areas of the dairy, and the amount of contaminated material contained on equipment. Mechanical farm equipment, such as front-end loaders and tractors, are commonly used to remove manure slurry from pens. The wheels, undercarriage, and surfaces of buckets and blades become heavily contaminated with manure solids, which are not often cleaned. Farm equipment used to move manure may not be restricted to the pens or manure storage area, and may be driven around the dairy yard and along common driveways. As a result of these practices, it is likely that manure slurry/environmental contamination removed from pens and alleyways will become deposited on environmental surfaces throughout the farm property, including the driveway and parking area adjacent to the milk house.

As discussed in Appendix B Environmental Persistence, the environmental factors that favor survival of virus are moist conditions, a neutral pH, and low temperature. Virus excreted during the preclinical and acute clinical phases can survive in the environment for weeks, or even months. Survival of the virus depends on the nature of the medium (secretions and excretions), the initial concentration of virus in the material, and the virus strain, RH, pH, and temperature—

and therefore is highly variable under field conditions. Residual virus may be remarkably resistant, especially in the presence of high concentrations of organic material (Alexandersen et al., 2003c).

10.2 Direct Routes for Contamination of the Hauler and Tanker

Likelihood of FMDv contaminated environmental media that leaves an infected but undetected farm, results in contamination of the environment, hauler, and tanker at doses sufficient to cause disease in susceptible livestock.

10.2.1 Hauler and Tanker Cab Route

Likelihood of hauler and tanker cab becoming contaminated by environmental media

The hauler‘s clothing, boots, and hands will become contaminated by handling equipment and moving in areas inside and outside of the milk house. The hauler’s boots may become contaminated with manure, dust, other soils, and debris that may adhere to the boots from areas around the milk house. The fraction of environmental media, or other soils, adhering to the driver’s boots, is proportional to the extent of contamination of the yard with manure, and would be highly variable among dairy operations. Current industry practices require that the ground around the milk house be kept relatively free of organic matter—reducing the overall likelihood of contaminating the driver’s boots. There would also be some mixing of the manure slurry on the boot with soil or dust, potentially reducing the FMD viral titer retained on the boot.

Data on experimental soil track-in-rates via shoes is beneficial in understanding how various factors can impact contamination of the driver’s shoes through this pathway. The mechanical transport of wet and dry soil from outdoor to indoor environments and the estimated soil deposition and dispersion rates was evaluated by (Hunt et al., 2006). Deposition tests involved two shoe sole types: a smooth leather sole and a rubber sole with fine tread. Despite the rapid drop off in the mass of wet soil deposition in the first 4 strides across a tile floor, a substantial proportion (between 34 and 65 percent) of the 2 grams of wet soil (mud) initially applied to the toe area of a shoe remained on the shoe by the 5^{th} stride. The wet soil rapidly dried on the sole, and the authors concluded that although this mass of soil was no longer subject to immediate deposition, it may pose a deposition risk at a later time.

Presumably, a fraction of the manure slurry on the sole would be transferred to the cab interior with further drying and flexing of the sole and abrasion with the floorboard of the cab (Hunt et al., 2006). Mechanical interactions between the footwear, soil, and flooring would result in redistribution of the contaminant on the floor of the cab (Hunt et al., 2006). Some mixing with soil already present on the floor would likely result in some reduction in FMD viral titer in dirt on the cab floor. The driver’s clothing could also become contaminated with dust, mud, or environmental media containing FMD virus; either from the contaminated cab interior or through

milk pumping practices such as handling of the exterior surfaces of the transfer hose that contact soils outside of the milk house. On occasion, the hauler may enter the milking parlor where his clothing and boots can become contaminated with aerosols, dust, or urine/manure from milking operations and cattle activity.

Current on-farm practices, and biosecurity measures, were also considered when estimating the likelihood of the truck driver being contaminated with FMDv. No nationally accepted biosecurity, or other risk measures for FMDv in a control area, have been specified at the time of this writing. The implementation and extent of on-farm biosecurity measures among companies that transport raw milk is highly variable. Haulers are not required to disinfect boots or don disposable footwear, wear clean or disposable coveralls, or use other personal protective gear such as gloves.

Likelihood Estimate: The likelihood of the hauler and tanker cab becoming contaminated by environmental media is moderate to high.

10.2.2 External Tanker Surface Route

Likelihood of external contamination of the tanker (includes storage compartments and transfer hose) by infectious environmental media.

The exterior surface of the vehicle represents another potential pathway of FMDv transmission. After entering the farm gate, milk tankers traverse common driveways used by farm equipment in order to access the milk house. The tires, wheel wells, and undercarriage of the tanker may become soiled to varying degrees with dust, mud, and manure, depending on the weather conditions. The percentage of manure slurry potentially on the tanker will be proportional to the contamination present on the path taken by the tanker to access the milk house. The degree of contamination will vary among dairy operations depending on the level of road maintenance, proximity to pens, and cleanliness of the operation. Although we lack data on the quantity of soil and manure that might adhere to the exterior surfaces of a contaminated milk tanker, we reason that the FMD viral titer in manure slurry contaminating the vehicle’s exterior will not be significantly diluted due to mixing with mud or dirt.

Connection of the transfer hose to the bulk tank will result in contact with the ground outside of the milk house as well as the floor inside of the milk house. Farm personnel walking through the milk house will potentially leave infectious fomites on the flooring and grounds of the milk house. Environmental contamination in both areas may be transferred to the exterior surface of the hose. Replacement of the transfer hose in the storage compartment after pumping will lead to transfer of hose contamination to the storage surfaces.

Truck tires, wheel wells, and undercarriage tanker contamination are a potential transmission pathway for FMDv, as was noted in the 2007 Surrey UK outbreak (Ellis-Iversen et al., 2011).

Treated FMDv in sewage effluent was transported on vehicle tires, and possibly other truck parts, for over 3 miles to the index farm. The trucks did not enter the farm, but drove on a road next to the farm. It was postulated that flooding on the road, along with cross traffic by farm equipment, carried the virus into the pasture where cattle were grazing (See Historical Outbreak Chapter 6 for description). In another study, Amass et al., (2003b) observed that truck tires contaminated under natural field conditions were potential mechanical vectors for the transmission of bacterial swine diseases. A mixture of gravel and soil was visible on the 3/4 ton pickup truck after driving under field conditions similar to those on a farm. The median number of aerobic bacterial counts cultured from a 4.02 cm^2 area of the tire footprint ranged from 1.55 x 10^5 to 3.31 x 10^5. Median bacterial counts from the tread groove ranged from 3.28 x 10^5 to 3.39 x 10^5 (0.855 cm^3). Bacterial numbers on tires varied with temperature and moisture. Driving along an asphalt road for 30 seconds reduced the bacterial count by as much as 1 log in some cases. The sidewalls and other surfaces of the wheel were not sampled so it is ambiguous as to whether the same degree of mud, soil, and bacterial contamination found on the tread surface would be found on the sidewalls of the tire, or the wheel rim, under similar conditions. The research indicated that microorganisms can easily survive and be transported on vehicle tires.

Based on the above information, it is reasonable to assume FMDv would be present in contaminated environmental media on areas of the tire and wheels other than the tread surface, assuming appropriate climatic conditions are present. The estimated pathogen load on the tire sidewalls, or metal rim, may not be significantly reduced by driving over dry pavement. Although the total quantity of soil and mud on the tire sidewall or wheel might be reduced while driving, the FMD viral titer in the remaining fraction of soil on the tires or wheels is not likely to be significantly reduced after leaving the farm.

Likelihood Estimate: Likelihood of external truck contamination (including transfer hose and storage compartments) by FMDv infectious environmental media is moderate to high.

10.3 Direct and Indirect Routes for Environmental Contamination

10.3.1 Hauler and Tanker Route

Likelihood of contaminated environmental media being deposited on an uninfected farm by the hauler and tanker directly.

The pathways by which the hauler and tanker were contaminated on the infected farm are also the routes by which environmental contamination can be transferred onto the uninfected farm. Loss of contaminated environmental media from the tanker to the ground/driveway will depend on weather conditions, the amount of media adhering to the tanker, and the road conditions that the tanker traverses. Loss of infectious materials from tires and undercarriage is a major concern, due to the amount of dirt/mud and manure that can be transported on a vehicle. The 2007 Surrey, United Kingdom outbreak indicates that small amounts of virus can be transported for long

distances. External contamination of the transfer hose will serve to release contamination onto the ground in front of the milk house and on the floor inside the milk house.

The driver's clothing has the potential to act as a vehicle for the spread of FMD to susceptible cattle. There are relatively few studies that have examined the survival and transmission of viruses directly from clothing (Bloomfield, 2011). The quantity of contaminated material that might adhere to the driver's clothing or shoes will vary depending on factors such as the season of the year, the type of soil around the dairy, and the type of sole on the shoe. Persistence of viruses on clothing varies depending on the type of fabric, the relative humidity, and the method of exposure (Bloomfield, 2011). Spilt milk on the hauler's pants and shirt would probably lead to contamination of his hands. Any surface that is touched could lead to transfer of the virus. Manure on the hauler's shirt and clothing would be another route for direct transfer of infectious material to surfaces that are contacted. In general, it is probably the contamination of the hauler's hands and boots that would serve as the most effective routes for virus transfer.

The virus will be transferred by the hauler and transfer hose into areas where cross-traffic with farm personnel occurs, such as the milk house and adjacent rooms. The hauler must transfer virus to surfaces and areas where farm personnel have direct contact. Farm personnel routinely walk through the milk house during pumping activities (personal observation by Risk Analyst, 2011). The hauler may, on occasion, enter the milking parlor or other adjacent rooms to find personnel. He could track in mud/manure on his boots and clothing that is transferred to surfaces (fomites) where contact by the farm personnel can result in contamination of the personnel's hands, clothing, and boots.

Likelihood Estimation: Likelihood of direct release of environmental media contamination from external surfaces of the tanker and hauler's clothing, boots and hands on an uninfected farm is moderate to high.

10.3.2 People, Vehicles, or Farms via Roadway Travel and other Stops Route

Likelihood of indirect cross-contamination of people, vehicles, or farms via deposition of environmental media from roadway travel or other stops.

As discussed above, any place that the hauler or tanker travels could lead to loss of infectious materials to roadways or driveways during travel, or flooring of buildings where the hauler makes a stop. Transfer of contamination to the grounds and floor of the processing plant will include the bays, waiting room, lab, and bathrooms. The transfer of fomites to common surfaces will result in contamination of boots, clothing, and hands of a new hauler. Cross contamination of tanker tires and undercarriage can occur on plant driveway, scale house, and unloading bays.

Likelihood Estimate: Likelihood of indirect cross contamination via deposition and tracking of media by people, vehicles, or farms by the hauler and tanker is moderate to high.

10.4 Overall Risk

Risk that transport of environmental contamination and infectious milk from an infected but undetected farm to another premises result in exposure of susceptible livestock.

Once the hauler and tanker have deposited contaminated environmental media and spilled milk either inside or outside of the milk house (or both), it will be the farm personnel, equipment, vehicles, and weather events that bring the virus into contact with susceptible livestock. The hauler does not have direct contact with livestock; however, they may transfer virus to surfaces and areas where farm personnel have contact. For example, the hauler may occasionally enter the milking parlor where they can track in mud/manure on their boots and also touch metal framework within the parlor. Transfer of virus to these surfaces could result in contamination of the milker's hands, clothing, and boots. Handling of the animals during milking and cleaning of the parlor with high-pressure spray hoses could lead to aerosolization of the virus that can have contact with livestock. Farm personnel also walk into pens and alleyways with contaminated boots. Virus could be aerosolized or have contact with abraded areas on the feet of cattle.

Honhold et al.(2004a) states that there is a lack of exact understanding of how virus is transferred once it is on a farm (Honhold et al., 2004a). Based on information contained in this risk assessment, including the virus properties of environmental persistence, resistance to inactivation, and protective properties of virus within milk, we generated a list of potential routes for exposure of livestock. These routes could result in small viral dose exposure and development of disease. Direct contact with livestock by farm personnel using equipment on animals, and directly handling of animals, will be a primary route for transfer of the virus. After direct contact, the most significant route for cattle exposure is via aerosolization and inhalation of virus. **Figure 4** presents potential routes for transporting the virus into animal areas from contamination and fomites that entered the farm through the transport of raw milk.

Figure 4 presents the idea that any farm activities, persons, vehicles, or weather event that can aerosolize environmental contamination/spilled infectious milk may result in exposure of cattle and development of disease. For example, wind and rain events that aerosolize virus—or move soils/environmental contamination to pens by surface run-off—can lead to exposure. Use of high-pressure spray devices within the milk house and milking parlor may aerosolize the virus, or move it to surface where cattle have contact. Mechanical transfer of the virus by people and vehicles, such as feed trucks or tractors, can lead to transport of the virus into pens and aerosolization of virus.

Risk Estimate: Risk that transport of environmental contamination and spilled infectious milk to other uninfected premises will result in exposure of susceptible livestock is rated as moderate to high.

10.5 Chapter Conclusion

In summary, the likelihood that contamination of the milk hauler and/or the tanker by FMDv contaminated media will result in disease in susceptible livestock is rated as *moderate to high*. There is a known association of vehicles and people in the spread of disease, but the routes for transfer of the virus once on farm are not clearly known. Based on UK outbreak information, once animal movements are stopped, disease spread occurs through the farm gate. During the 2001 outbreak in Cumbria, DEFRA imposed enhanced and enforced biosecurity practices for external cleaning of milk tankers, including the fitting of pressure washers to the vehicles. These C&D activities were felt to be responsible for ending the epidemic in three areas. In the 2007 United Kingdom outbreak, contamination of tires and undercarriage of trucks hauling virus contaminated soils to a landfill—was attributed to the infection of the index farm. The trucks travelled on a road adjacent to the farm, but did not enter the farm. FMDv can be transported by soils, clothing/boots, and hands, which can subsequently be transferred to personnel and equipment that may have direct contact livestock. Due to this lack knowledge on how the virus actually moves within the environment, along with the massive amounts of infectious manure that can be produced during the preclinical stage of disease, the likelihood must be considered moderate to high. Once on-farm mitigation measures are proposed, these estimates will be re-evaluated.

11. Overall Conclusions

The transport of raw milk during an FMD outbreak poses a moderate to high risk for transfer of virus from infected but undetected farm(s) to uninfected farms via the movement of FMDv contaminated milk and environmental media by the tanker and hauler. External contamination of the tanker (wheel wells, undercarriage, storage compartments, and transfer hose) and contaminated materials carried by the hauler on his clothing, hands, or boots will be the primary sources for transfer of virus from an infected but undetected farm.

FMDv will be present in raw milk and environmental media before disease is detected at concentrations that pose a risk to cattle via inhalation exposure. Exposure to 0.1 to 0.01 ml of FMDv contaminated milk that is aerosolized could contain enough virus to establish infection in a susceptible cow. Contaminated environmental media will pose a risk due to the large volume of contaminated material that will be produced, ranging from 20,370 to 81,000 lbs. of contaminated manure produced daily by a 1,000 head dairy herd. The viral concentrations in environmental media are expected to be slightly lower than the viral titers in milk.

The average time to detect disease post-infection in all farms sizes combined is 5-6 days, with a range of 2 to 9 days. Regardless of farm size, approximately 62 percent of the herd is estimated to be infectious (preclinical and clinical) by the time FMD is detected in the herd. Detection is expected to occur when 5 to 10 percent of the herd is showing clinical signs. Due to the lag time to detect disease on susceptible farms, there will l be significant concentrations of FMDv in environmental media and raw milk for several days prior to detection of disease. This material will potentially transported off-farm by the tanker and other farm vehicles.

The key results of this assessment for risk associated with contaminated milk and environmental media are summarized below:

- The overall likelihood of bioaerosols emanating from a tanker and spreading infectious virus through milk collection and transport activities is estimated as low to very low.
- Likelihood of hauler and truck cab contamination by spilled milk at an infected but undetected farm is moderate to high.
- The likelihood of external tanker, storage compartments, and transfer hose contamination by spilled milk at an infected farm is moderate to high.
- The likelihood that viable virus will remain in the tanker after unloading milk but not undergoing the CIP process will result in release of virus on an uninfected farm is negligible to low.
- The likelihood that viable virus will remain in the tanker after unloading milk and after undergoing the CIP process will result in release of virus on an uninfected farm is negligible.

- The likelihood of a direct release of virus via milk spillage from the transfer hose on an uninfected farm is moderate to high.
- The likelihood of direct release of virus from spilled milk contamination of the external tanker surfaces or hauler's clothing, boots, or hands is moderate to high.
- Likelihood of accidental milk spillage resulting in cross-contamination of a person, vehicle, or farm from travel on common roadways or other stop is low.
- The likelihood of the hauler and cab being contaminated by environmental media is moderate to high.
- The likelihood of the external tanker contamination (including storage compartments and transfer hose) by environmental media is moderate to high.
- Likelihood of direct release of environmental media contamination from external surfaces of the tanker and hauler's clothing, boots, and hands on an uninfected farm is moderate to high.
- Likelihood of cross-contamination of people, vehicles, or farms via deposition of environmental media on common roads and other surfaces from external contamination of the tanker and hauler is moderate to high.

Conclusion

Based on the estimated concentrations of virus in contaminated milk and environmental media that markedly exceed the minimum infectious doses for inhalation exposure in cattle, and the pathway evaluations which indicated moderate to high likelihood for transferring infectious virus via tanker activities and milk movement, the risk that the movement of raw milk from a Grade "A" dairy cattle farm to processing into, within, and outside of a control area during an FMD outbreak in the United States will result in infection of susceptible animals on other premises is moderate to high

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13. References

Aggarwal, N., Zhang, Z., Cox, S., Statham, R., Alexandersen, S., Kitching, R. P., et al. (2002). Experimental studies with foot-and-mouth disease virus, strain O, responsible for the 2001 epidemic in the United Kingdom. Vaccine, 20(19-20), 2508-2515.

Alexandersen, S., 2005. Virus Inactivation Kinetics. FAO Appendix 23 accessed at: http://www.fao.org/ag/againfo/commissions/docs/research_group/germany05/App23.pdf

Alexandersen, S., Kitching, R.P., Mansley, L.M., Donaldson, A.I., 2003a. Clinical and laboratory investigations of five outbreaks of foot-and-mouth disease during the 2001 epidemic in the United Kingdom. Vet Rec 152, 489-496.

Alexandersen, S., Quan, M., Murphy, C., Knight, J., Zhang, Z., 2003b. Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. J Comp Pathol 129, 268-282.

Alexandersen, S., Zhang, Z., Donaldson, A.I., Garland, A.J., 2003c. The pathogenesis and diagnosis of foot-and-mouth disease. J Comp Pathol 129, 1-36.

Alexandersen, S., Mowat, N., 2005. Foot-and-mouth disease: host range and pathogenesis. Curr Top Microbiol Immunol 288, 9-42.

Amass, S.F., Pacheco, J.M., Mason, P.W., Schneider, J.L., Alvarez, R.M., Clark, L.K. and Ragland, D. 2003a. Procedures for preventing the transmission of foot-and-mouth disease virus to pigs and sheep by personnel in contact with infected pigs. Vet Rec **153**(5): 137-40

Amass, S.F., Schneider, J.L., Ragland, D., Hill, M.A., 2003b. Pilot studies to evaluate the efficacy of a truck-mounted tire sanitizer system. Journal of Swine Health and Production 11, 277-283.

Anonymous, 1968. Report of the Committee of Inquiry of Foot and Mouth Disease. In: Ministry of Agriculture, F.F., Her Majesty's Stationery Office (Ed.), Vol. Part 1. pp. 56-57.

Bachrach, H.L., Breese, S.S.J., Callis, J.J., Hess, W.R., Patty, R.E., 1957a. Inactivation of foot-and-mouth disease virus by pH and temperature changes and by formaldehyde. Proc Soc Exp Biol Med 95, 147-152.

Bachrach, H.L., Callis, J.J., Hess, W.R., Patty, R.E., 1957b. A plaque assay for foot-and-mouth disease virus and kinetics of virus reproduction. Virology 4, 224-236

Bachrach, H.L., 1961. Thermal degradation of foot-and-mouth disease virus into infectious ribonucleic acid. Proc Soc Exp Biol Med 107, 610-613.

Bachrach, H.L., 1968. Foot-and-mouth disease. Annu Rev Microbiol 22, 201-244.

Baltimore, D. 1969. The replication of picornaviruses,. In H. B. Levy (ed.), The biochemistry of viruses. Marcel Dekker, New York.. p.101-176.

Blackwell, J.H., Hyde, J.L., 1976. Effect of heat on foot-and-mouth disease virus (FMDV) in the components of milk from FMDV-infected cows. J Hyg (Lond) 77, 77-83.

Blackwell, J.H., McKercher, P.D., Kosikowski, F.V., Carmichael, L.E., Gorewit, R.C., 1982. Concentration of foot-and-mouth disease virus in milk of cows infected under simulated field conditions. J Dairy Sci 65, 1624-1631.

Blackwell, J.H., McKercher, P.D., Kosikowski, F.V., Carmichael, L.E., Gorewit, R.C., 1983. Physicochemical transformation of milk components and release of foot-and-mouth disease virus. J Dairy Res 50, 17-25.

Bloomfield, S.F. 2011 The infection risks associated with clothing and household linens in home and everyday life settings,

and the role of laundry. In. Simmons College, Boston, MA.

Boone, S.A., Gerba, C.P., 2007. Significance of fomites in the spread of respiratory and enteric viral disease. Appl Environ Microbiol 73, 1687-1696.

Burrows, R., 1968. Excretion of foot and mouth disease virus prior to the development of lesions. Veterinary Record 83, 387-388.

Burrows, R., Mann, J.A., Greig, A., Chapman, W.G., Goodridge, D., 1971. The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland. J Hyg (Lond) 69, 307-321.

Burrows, R., J.A. Mann, A.J. Garland, A. Greig, and D. Goodridge 1981 The pathogenesis of natural and simulated natural foot-and-mouth disease infection in cattle. J. Comp. Pathol. 91: 599-609.

Carpenter, T.E., Thurmond, M.C., Bates, T.W., 2004. A simulation model of intraherd transmission of foot and mouth disease with reference to disease spread before and after clinical diagnosis. J Vet Diagn Invest 16, 11-16.

Coetzer, J., Thomsen, G., Tustin, R. and Kriek, N., *Foot and Mouth Disease*. Infectious Diseases of Livestock with Special Reference to Southern Africa. 1994, Cape Town: Oxford University Press.

Cottral, G.E., 1969. Persistence of foot-and-mouth disease virus in animals, their products and the environment. Bull Off Int Epizoot 70, 549-568.

Cottral, G.E., Bachrach, H.L., 1968. Food-and-mouth disease viremia. Proc Annu Meet U S Anim Health Assoc 72, 383-399.

Cox, C.S. 1989 Airborne bacteria and viruses. Science Progress, 73 (292 Pt 4): 469-499.

Cox, C. and Wathes, C., 1995. Bioaerosols Handbook. New York: Lewis Publication.

Daggupaty, S.M., Sellers, R.F., 1990. Airborne spread of foot-and-mouth disease in Saskatchewan, Canada, 1951-1952. Can J Vet Res 54, 465-468.

Davis, D. QA Manager, Dairy Farmers of America - Ft. Morgan CO.

Dawson, P.S., 1970. The involvement of milk in the spread of foot-and-mouth disease: an epidemiological study. Vet Rec 87, 543-548.

DBL Tank, 2011. Dry Bulk and Liquid Tank Services, Inc. from http://www.dbltank.com/

Dekker, A., 1998. Inactivation of foot-and-mouth disease virus by heat, formaldehyde, ethylene oxide and gamma radiation. Vet Rec 143, 168-169.

DeLeeuw, P., Bekkum, J.V., 1979. Report of the session of the research group of the standing technical committee of the European Commission for the control of foot and mouth disease. Linholm, Denmark.

De Leeuw, P.W., Tiessink, J.W., van Bekkum, J.G., 1980. Aspects of heat inactivation of foot-and-mouth disease virus in milk from intramammarily infected susceptible cows. J Hyg (Lond) 84, 159-172.

Department for Environmental Food and Rural Affairs, 2007. FMD Epidemiology Report 30/09/07. http://archive.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/fmd/2007/index.htm.

Dietz, K., Schenzle, D 1985 Proportionate mixing models for age-dependent infection transmission. J. Math. Bioi. 22, 1 17-120.

Dimmock, N.J., 1967. Differences between the thermal inactivation of picornaviruses at "high" and "low" temperatures. Virology 31, 338-353.

Donaldson, A.I., Ferris, N.P., 1975. The survival of foot-and-mouth disease virus in open air conditions. J Hyg (Lond) 74, 409-416.

Donaldson, A., 1986. Aerobiology of foot and mouth disease (FMD): an outline and recent advances. Rev Sci Tech OIE 5, 315-321.

Donaldson, A.I. 1987 Foot-and-Mouth disease: the principal features. Irish Vet. J. 41, 325-327.

Donaldson, A.I., 1997. Risks of spreading foot and mouth disease through milk and dairy products. Rev Sci Tech 16, 117-124.

Donaldson, A.I., Gloster, J., Harvey, L.D., Deans, D.H., 1982. Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey and the Isle of Wight in 1981. Vet Rec 110, 53-57.

Donaldson, A.I., Gibson, C.F., Oliver, R., Hamblin, C., Kitching, R.P., 1987. Infection of cattle by airborne foot-and-mouth disease virus: minimal doses with O1 and SAT 2 strains. Res Vet Sci 43, 339-346.

Donaldson, A.I., Alexandersen, S., Sorensen, J.H. and Mikkelsen, T. 2001. Relative risks of the uncontrollable (airborne) spread of FMD by different species. Vet Rec **148**(19): 602-604.

Donaldson, A.I., Alexandersen, S., 2001. Relative resistance of pigs to infection by natural aerosols of FMD virus. Vet Rec 148, 600-602.

Donaldson, A.I. and Alexandersen, S., 2002. Predicting the spread of foot and mouth disease by airborne virus. Rev Sci Tech 21, 569-575.

Donaldson, A.I. and R.P. Kitching 1989 Transmission of foot-and-mouth disase by vaccinated cattle following natural challenge. Res vet Sci 46, 9-14.

Dungan, R.S., 2010. BOARD-INVITED REVIEW: fate and transport of bioaerosols associated with livestock operations and manures. J Anim Sci 88, 3693-3706.

Ellis-Iversen, J., Smith, R.P., Gibbens, J.C., Sharpe, C.E., Dominguez, M., Cook, A.J., 2011. Risk factors for transmission of foot-and-mouth disease during an outbreak in southern England in 2007. Vet Rec 168, 128.

FDA US Department of Human Health Services, 2009. Grade A pasteurized milk ordinance. retrieved, from http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/MilkSafety/NationalConferenceonInterstateMilkShipmentsNCIMSModelDocuments/default.htm .

Ferris, N.P., Philpot, R.M., Oxtoby, J.M., Armstrong, R.M., 1990. Freeze-drying foot-and-mouth disease virus antigens. I. Infectivity studies. J Virol Methods 29, 43-52.

Garland, A., 1974. The inhibitory activity of secretions in cattle against FMDV. Thesis Submitted for degree of Doctor of Philosophy, University of London, 1-262.

Geering, W.A., Lubroth, J., 2002. Food and Agriculture Organization of the United Nations.

Gibbens, J.C., Sharpe, C.E., Wilesmith, J.W., Mansley, L.M., Michalopoulou, E., Ryan, J.B., Hudson, M., 2001. Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: the first five months. Vet Rec 149, 729-743.

Gibson, B., Field Manager, Tillamook Cheese Corp. 2012 personal commuication.

Gilbert, Y., Duchaine, C., 2009. Bioaerosols in Industrial Environments: a review. Canadian Journal of Civil Engineering 36, 1873-1886.

Gloster, J., Alexandersen, S., 2004. New Directions: Airborne transmission of foot and mouth disease virus. Atmospheric Environment 38, 503-505.

Gloster, J., Freshwater, A., Sellers, R.F., Alexandersen, S., 2005. Re-assessing the likelihood of airborne spread of foot-and-mouth diseaseat the start of the 1967-1968 UK foot-and-mouth disease epidemic. Epidemiol. Infect. 133, 767-783.

Gloster, J., Ryan, E., Wright, C., Doel, C., Parida, S., Cox, S., Barnett, P., Schley, D., Gubbins, S., Paton, D., 2008. Foot-and-mouth disease: How much airborne virus do animals exhale? Vet J.

Gloster, J., Williams, P., Doel, C., Esteves, I., Coe, H., Valarcher, J.F., 2007. Foot-and-mouth disease - quantification and size distribution of airborne particles emitted by healthy and infected pigs. Vet J 174, 42-53.

Gomes I, Ramalho AK, Auge de Mello P.1997 Infectivity of foot-and-mouth disease virus: contact transmission between cattle and buffalo (*Bubalus bubalis*) in the early stages of infection. The Veterinary Record. 140:43–47.

Graves, R., 1994. Traffic Patterns and Barn Layouts. In: Expansion Strategies for Dairy Farms-Facilities and Financial Planning, Ellicott, MD.

Graves, R., Light, R., 1980. Animal Handling and Movement. In: National Milking Center Design Conference, Northeast Regional Agricultural Engineering Service, pp. 224-252.

Grinshpun, S., Buttner, M., Willeke, K., 2007. Sampling for airborne microorgansims. In, Manual for Environmental Microbiology. ASM Press, Washington, DC.

Haas, B., Ahl, R., Bohm, R., Strauch, D., 1995. Inactivation of viruses in liquid manure. Rev. Sci. tech. Off. int. Epiz. 14, 435-445.

Hardy, R., Schilling, K., Fromm, J., Dai, X., Cook, M., 2006. Technical backgroung document: Microbial risk assessment and fate and transport modeling of aerosolized microorganisms at wastewater land application facilities in Idaho. In. Idaho Department of Environmental Quality.

Harper, G., 1968. Tests made to measure a number of microorganisms escaping from the vacuum pump outlet of a bulk collection milk tanker. In, Vol. Technical Note No 15. Ministry of Defence, Microbial Research Establishment, Porton Down, Salisbury, Wiltshire. pp. 1-5 (as cited in Donaldson 1997).

Hedger, R.S., Dawson, P.S., 1970. Foot-and-Mouth Disease Virus in Milk: An Epidemiological Study. The Veterinary Record, 186-213.

Honhold, N., 2011. Foot and Mouth Disease. University of Minnesota. 2011: Professional Presentation.

Honhold, N., Taucher, T., Taylor, N., 2004a. The involvement of milk tankers in the spread of foot and mouth disease in Cumbria, 2001. In. The University of Reading.

Honhold, N., Taylor, N.M., Mansley, L.M., Paterson, A.D., 2004b. Relationship of speed of slaughter on infected premises and intensity of culling of other premises to the rate of spread of the foot-and-mouth disease epidemic in Great Britain, 2001. Vet Rec 155, 287-294.

Hugh-Jones, M.E., 1976. A simulation spatial model of the spread of foot-and-mouth disease through the primary movement of milk. J Hyg (Lond) 77, 1-9.

Hunt, A., Johnson, D.L., Griffith, D.A., 2006. Mass transfer of soil indoors by track-in on footwear. Sci Total Environ 370, 360-371.

Hyde, J.L., Blackwell, J.H., Callis, J.J., 1975. Effect of pasteurization and evaporation on foot-and-mouth disease virus in whole milk from infected cows. Can J Comp Med 39, 305-309.

Hyslop, N.S., 1965. Secretion of Foot and Mouth Disease Virus and antibody in the saliva of infected and immunized cattle. J Comp Pathol 75, 111-117.

Hyslop, N.S., 1970. The epizootiology and epidemiology of foot and mouth disease. Adv Vet Sci Comp Med 14, 261-307.

Iowa State University, The Center for Food Security and Public Health, 2007. Foot and Mouth Disease website *www.cfsph.ia**state**.edu/DiseaseInfo/disease*

Jones, A.M., Harrison, R.M., 2004. The effects of meteorological factors on atmospheric bioaerosol concentrations--a review. Sci Total Environ 326, 151-180.

Juleff, Nick FMD expert, Institute for Animal Health, Pirbright UK, personal communication 2012.

Kitching, R.P., 1998. A recent history of foot-and-mouth disease. J Comp Pathol 118, 89-108.

Kitching, R.P., 2002. Identification of foot and mouth disease virus carrier and subclinically

infected animals and differentiation from vaccinated animals. Rev Sci Tech 21, 531-538.

Kitching, R.P., Hutber, A.M., Thrusfield, M.V., 2005. A review of foot-and-mouth disease with special consideration for the clinical and epidemiological factors relevant to predictive modelling of the disease. Vet J 169, 197-209.

Kitching, P., Hammond, J., Jeggo, M., Charleston, B., Paton, D., Rodriguez, L. and Heckert, R. 2007 "Global FMD control--is it an option?" Vaccine **25**(30): 5660-4.

Knowles, N.J., Samuel, A.R., Davies, P.R., Kitching, R.P., Donaldson, A.I., 2001. Outbreak of foot-and-mouth disease virus serotype O in the UK caused by a pandemic strain. Vet Rec 148, 258-259.

Krug, P.W., Lee, L.J., Eslami, A.C., Larson, C.R., Rodriguez, L., 2011a. Chemical disinfection of high-consequence transboundary animal disease viruses on nonporous surfaces. Biologicals 39, 231-235.

Krug, P.W., Larson, C.R., Eslami A.C., Rodriguez, L. 2011b Disinfection of foot-and-mouth disease and African swine fever viruses with citric acid and sodium hypochlorite on birch wood carriers. Vet. Microbiol.

Krug, P., 2012. Reseach Microbiologist, FAD research Unit, Plum Island, Agricultural Research Service-USDA. Personal Communication

Lighthart, B., 1994. Physics of microbial bioaerosols. In, Atmospheric Microbial Aerosols: Theory and Applications, New York, NY.

Lighthart, B., 2000. Mini-review of the concentration variations found in the alfresco atmospheric bacterial populations. Aerobiologia 16, 7-16.

Lombard, J. 2012 Dairy Specialist USDA:APHIS:VS:CEAH:NAHMS, Personal Communication.

Macher, J., Ammann, H., Milton, D., Burge, H., Morey, P., 1999. Bioaerosols: Assessment and Control. In: American Conference of Governmental Industrial Hygienists, Cincinnati, OH

Mohr, A., 2005. Aerosol (Aerobiology, Aerosols, Bioaerosols, Microbial Aerosols). In: Pilch, R., Zilinskas, R. (Eds.), Aerosol Vol. Encyclopedia of Bioterrorism Defense.

Mohr, A., 2007. Fate and transport of microorganisms in air. In, Manual for Environmental Microbiology. ASM Press, Washington, DC.

Mohr, A., 2011. Special Programs Division, US Army Dugway Proving Ground, Personal Communicaton.

Muilenberg, M., 1995. The outdoor aerosol. In, Bioaerosols. Lewis Publishers, Boca Raton, FL.

NAHMS, 2007. Part III Reference of dairy cattle health and management practices in the United States. In: National Animal Health Monitoring System (Ed.)

National Center for Animal Health Emergency Management, 2011. Foot and Mouth Disease Response Plan: The Red Book. USDA APHIS VS.

OIE World Organisation for Animal Health FMD May 2012 http://www.oie.int/animal-health-in-the-world/fmd-portal/

Orsel, K., de Jong, M.C., Bouma, A., Stegeman, J.A., Dekker, A., 2007. The effect of vaccination on foot and mouth disease virus transmission among dairy cows. Vaccine 25, 327-335.

Orsel, K., Bouma, A., Dekker, A., Stegeman, J.A., de Jong, M.C., 2009. Foot and mouth disease virus transmission during the incubation period of the disease in piglets, lambs, calves, and dairy cows. Prev Vet Med 88, 158-163.

Orsel, K., Dekker, A., Stegeman, J.A., De Jong, M.C., Bouma, A., 2010. Different infection parameters between dairy cows and calves after an infection with foot-and-mouth disease virus. Vet J. Oct; 186 (1): 116-8.

Parker, J., 1971. Presence and inactivation of foot-and-mouth disease virus in animal faeces. Vet Rec 88, 659-662.

Paton, D.J., Sumption, K.J. and Charleston, B. (2009). Options for control of foot-and-mouth disease: knowledge, capability and policy. Philos Trans R Soc Lond B Biol Sci **364**(1530): 2657-67.

Perez, A.M., Ward, M.P., Carpenter, T.E., 2004. Epidemiological investigations of the 2001 foot-and-mouth disease outbreak in Argentina. Vet Rec 154, 777-782.

Pillai, S.D., Ricke, S.C., 2002. Bioaerosols from municipal and animal wastes: background and contemporary issues. Can J Microbiol 48, 681-696.

Reid, S., Parida, S., King, D., Hutchings, G., Shaw, A., Ferris, N., Zhang, Z., Hillerton, J., Paton, D., 2004. Use of automated RT-PCR to detect FMDV in milk. FAO Appendix 43, 268-276.

Reid, S.M., Parida, S., King, D.P., Hutchings, G.H., Shaw, A.E., Ferris, N.P., Zhang, Z., Hillerton, J.E., Paton, D.J., 2006. Utility of automated real-time RT-PCR for the detection of foot-and-mouth disease virus excreted in milk. Vet Res 37, 121-132.

Ryan, E., Mackay, D., Donaldson, A., 2008. Foot-and-mouth disease virus concentrations in products of animal origin. Transbound Emerg Dis 55, 89-98.

Sahu, S.P. and Dardiri, A.H. 1979. Susceptibility of mink to certain viral animal diseases foreign to the United States. J Wildl Dis **15**(3): 489-94.

Salem, H., Gardner, D., 1994. Health aspects of bioaerosols. In, Atmospheric Microbial Aerosols: Theory and Applications. Chapman & Hall, New York, NY.

Sanson, R.L., 1994. The epidemiology of foot-and-mouth disease: implications for New Zealand. N Z Vet J 42, 41-53.

Sattar, S., Springthorpe, V., 1996. Transmission of viral infections through animate and inanimate surfaces and infection control through chemical disinfection. In: Hurst, C. (Ed.), Modeling disease transmission and its prevention by disinfection. Cambridge University Press, Cambridge, pp. 224-257.

Scott, F., Cottral, G., Galiliunas, P., 1966. Persistence of foot and mouth disease virus in external lesions and saliva of experimentally infected cattle. American Journal of Veterinary Research 27, 1531-1536.

Scudamore, J.M., Harris, D.M., 2002. Control of foot and mouth disease: lessons from the experience of the outbreak in Great Britain in 2001. Rev Sci Tech 21, 699-710.

Sellers, R.F., 1968 The inactivation of foot-and-mouth disease virus by chemical and disinfectants. Vet Rec 83: 504-506

Sellers, R.F., 1969 Inactivation of foot-and-mouth disease virus in milk. Br. Vet. J. 125:163-167.

Sellers, R., 1971. Quantitative aspects of the spread of foot and mouth disease. Vet. Bull 41, 431-439.

Sellers, R.F., Parker, J., 1969. Airborne excretion of foot-and-mouth disease virus. J Hyg (Lond) 67, 671-677.

Sellers, R.F, and Gloster, J., 2008. Foot-and-mouth disease: a review of intranasal infection of cattle, sheep and pigs. The Veterinary Journal 177, 159–168

Sigurdson, C., Cords, B., Fredell, D., 2004. Practical Hygiene and Disinfection on Dairy Farms. In, Minnesota Dairy Health Conference.

Sutmoller, P., McVicar, J.W., 1976. Pathogenesis of foot-and-mouth disease: the lung as an additional portal of entry of the virus. J. Hyg. (Camb.) 77, 235-243.

Tomasula, P.M., Kozempel, M.F., Konstance, R.P., Gregg, D., Boettcher, S., Baxt, B., Rodriguez, L.L., 2007. Thermal inactivation of foot-and-mouth disease virus in milk using high-temperature, short-time pasteurization. J Dairy Sci 90, 3202-3211.

United States Animal Health Association, 2008. Foreign Animal Diseases, 7th Edition. Boca Publications Group, Inc., Boca Raton, FL.

USDA NASS National Agricultural Statistics Service 2006, 2010 Milk Production Data www.nass.usda.gov.

USDA, 2012. Draft. Parameters used to simulate the spread of Foot and Mouth Disease in Texas using the North American Animal Disease Spread Model (NAADSM). In, USDA APHIS VS CEAH, Ft. Collins, CO.

USDA:APHIS:VS:CEAH, 2007. Foot and mouth disease: sources of outbreaks and hazard categorization of modes of virus transmission.

Valarcher, J.F., Leforban, Y., Rweyemamu, M., Roeder, P.L., Gerbier, G., Mackay, D.K., Sumption, K.J., Paton, D.J., Knowles, N.J., 2008. Incursions of foot-and-mouth disease virus into Europe between 1985 and 2006. Transbound Emerg Dis 55, 14-34.

Walker, J.L., P.W. deLeeuw, J.J. Callis J.V. van Bekkum. 1984 Thethermal death time curve for foot-and-mouth disease virus contained in primarily infected milk. J.Biol. Stand. 12:185-189.

Appendix A. Demographics of the U.S. Dairy Industry

Since 2001, the overall trend in the U.S. dairy industry has been towards an increasing concentration in the number of large-scale operations (premises with 500 or more head of milk cows) with a greater share of total milk cow inventory and total milk production. In 2009, farms with ≥ 500 head accounted for 5 percent of the total milk cow operations, 56 percent of milk cows, and 60 percent of milk production. The most dramatic increases occurred in the largest herd size of ≥ 2,000 head, which accounted for 1 percent of all operations, 30 percent of milk cows, and 31 percent of milk production. Milk production continues to shift to the western half of the United States, although the upper mid-west region has also experienced increasing milk production. **Table A-1** presents an overview of information on operations, cow inventory, and percent milk production by herd size.

Table A-1. Number of dairy operations, percent inventory, and percent production by size group. U.S., 2008-2009 (NASS 2010).

Head	Operations		Percent of Cow Inventory		Percent of Milk Production	
	2008	2009	2008	2009	2008	2009
1-29	21,300	20,400	1.8	1.8	1.2	1.2
30-49	11,900	11,500	5.1	4.9	3.9	3.8
50-99	17,800	17,300	13.1	13.0	11.5	11.4
100-199	8,700	8,600	12.5	12.4	11.8	11.6
200-499	3,950	3,850	12.6	12.3	13.1	12.5
500-999	1,720	1,700	12.5	12.5	12.5	12.6
1,000-1,999	900	910	13.1	13.3	15.5	15.7
2,000+	730	740	29.3	29.8	30.5	31.2
TOTAL	**67,000**	**65,000**	**100.0**	**100.0**	**100.0**	**100.0**

The number of U.S. milk-cow operations declined 33 percent from 97,460 to 65,000 operations from 2001 to 2009. During this time, milk production increased 15 percent from 165,332 million pounds (2001) to 189,320 million pounds (2009). Milk cow inventory showed an increase of 1 percent, from 9.1 to 9.2 million head from 2001 to 2009.

Although the number of milk cow operations has declined since 2001, the number of operations with 500 or more head has increased. From 2001 to 2009, the number of operations with 500 or more head increased 20 percent from 2,795 (2001) to 3,350 (2009). The operations with 2,000 or more head showed the greatest percentage change (128 percent) during this time increasing from 325 places (2001) to 740 places (2009). From 2001 to 2009, smaller operations, those with less than 500 head decreased 35 percent from 94,665 to 61,650 places. **Table A-2** presents production information for the top 10 dairy producing States.

Table A-2. Top 10 dairy producing States in 2009 (NASS 2010).

State	Production (Millions of Pounds)	Percent of Total U.S. Production	Number of Operations with Milk Cows (2007 data)	Number of Licensed Dairy Herds	Number of Cows (x1000)
CALIFORNIA	39,512	20.87%	2,200	1,820	1,796
WISCONSIN	25,239	13.33%	14,200	13,170	1,257
NEW YORK	12,424	6.56%	5,700	5,470	619
IDAHO	12,150	6.42%	810	600	550
PENNSYLVANIA	10,551	5.57%	8,300	7,400	545
MINNESOTA	9,019	4.76%	5,100	4,700	469
TEXAS	8,840	4.67%	1,300	650	423
MICHIGAN	7,968	4.21%	2,700	2,310	355
NEW MEXICO	7,904	4.17%	270	150	325
WASHINGTON	5,561	2.94%	820	470	240
TOTAL U.S. production	**189,320**	**185,656**	**69,995**	**54,942**	**9,201**

As large operations have become more numerous, the share of inventory held by large operations has also increased. In 2009, operations with ≥ 500 head (500 to 2,000+) accounted for 56 percent of the total milk-cow inventory, compared to 35 percent in 2001. Operations with ≥ 2,000 head accounted for 30 percent of inventory in 2009, up from 12 percent in 2001. Places with less than 500 head accounted for 44 percent of total milk cow inventory in 2009, down from 65 percent in 2001.

As with inventory, the share of milk production accounted for by large operations has steadily increased. Operations with ≥ 500 head accounted for nearly 60 percent of all milk produced in 2009, up from 39 percent in 2001. Milk production on operations with 2,000 or more head has increased from 13 to 31 percent from 2001 to 2009. Smaller operations continue to produce a smaller share of production. Operations with fewer than 500 head accounted for nearly 41 percent of milk production in 2009, down from 61 percent in 2001.

A strong increase in milk production from operations with ≥ 500 head (up 74 percent) substantially offset the production decline from operation with fewer than 500 cows, resulting in an overall production increase in 2009. The increased production from operations with ≥ 500 head is due to the increase in number of operations, greater share of inventory, and increased milk production per cow. **Table A-3** presents the production and herd size of farms by region of the United States.

Table A-3. Milk production and farm structure in major dairy States (NASS Farms, Land in Farms and Livestock Operations 2006).

State	Production (Billion pounds)	Herd size < 100 (Percent of State Production)	Herd Size > 499 (Percent of State Production)
Northeast	25.3	38.9	21.3
NY	12.0	28.5	31.0
PA	10.7	53.0	10.0
VT	2.6	26.0	29.0
E. Corn Belt	15.3	28.4	31.2
IN	3.3	29.0	43.0
MI	7.1	18.0	39.0
OH	4.9	36.0	23.0
Upper Midwest	31.8	48.2	15.6
MN	8.4	47.5	17.5
WI	23.4	45.0	19.0
Southwest	17.8	1.6	87.3
NM	7.6	0.2	98.0
AZ	3.7	0.5	98.0
TX	7.1	2.0	78.0
West	59.9	1.3	84.2
CA	38.8	0.5	88.0
CO	2.5	1.9	83.0
ID	10.9	2.0	89.0
OR	2.2	6.0	54.0
WA	5.5	2.4	70.0
16 major states	**150.1**	**20.4**	**54.0**
U.S. Total	**181.8**	**20.4**	**51.6**

Appendix B. Additional Hazard Identification Information

Agent and Host Range

It is important to distinguish the animals that play a role in the natural epidemiology of the disease from those that may play a role only under certain conditions, or cannot be excluded as being an epidemiological risk (Alexandersen and Mowat, 2005). Cattle, sheep, pigs, goats, and other farmed cloven hoofed animals (e.g., water buffalo) are the species of concern in the natural epidemiology of FMDv (Kitching et al., 2005, 2007).

Deer, camels, llamas, alpacas, Indian elephants, and wild animals of the order Artodactyla (even-toed ungulates; e.g., deer, elk, feral pig, pronghorn, bison, mountain sheep) fall into the category of animals which may contribute to viral transmission under certain conditions, or cannot be excluded as presenting some risk of transmission. These species do not appear to play an important role in the wild. However, they may have to be considered as a potential risk under crowded or farmed conditions, or in zoos, if they have contact with livestock. All animals can transfer the virus mechanically and become fomites, if they become contaminated and are in close contact with susceptible livestock — including highly resistant species such as horses and carnivores (Alexandersen, 2005; Alexandersen and Mowat, 2005).

A large range of other species including mice, guinea pigs, rabbits, cats, dogs, mink, monkeys, snakes, birds, chickens, and embryonated eggs have been infected with FMDv under experimental conditions (Cottral and Bachrach, 1968; Hyslop, 1970; Sahu and Dardiri, 1979). Infection of these species is possible, but is not likely to play a role under field conditions (Alexandersen and Mowat, 2005).

Geographic Distribution

The current global distribution of FMDv infection is maintained within three continental reservoirs in Asia, Africa, and South America and can be further subdivided into seven major virus pools of infection. Each of the pools contains at least three serotypes of virus and, because virus circulation is mainly within these regional reservoirs, strains have evolved which are specific to the region (Paton et al., 2009). An eighth pool of infection in Western Europe was present until the 1980's, but has been eradicated through preventive vaccination and zoo-sanitary measures (Valarcher et al., 2008; Paton et al., 2009). The European strains of type O and A are currently maintained only in parts of South America (Paton et al., 2009). **Figure B-1** shows the worldwide distribution of the virus strains. The current list of FMDv free countries is maintained by the World Organization for Animal Health (OIE) on their website (OIE, 2012). North America, New Zealand, Australia, Greenland, Iceland, and most of Europe are currently free of the disease. The last U.S. outbreak of FMDv occurred in 1929.

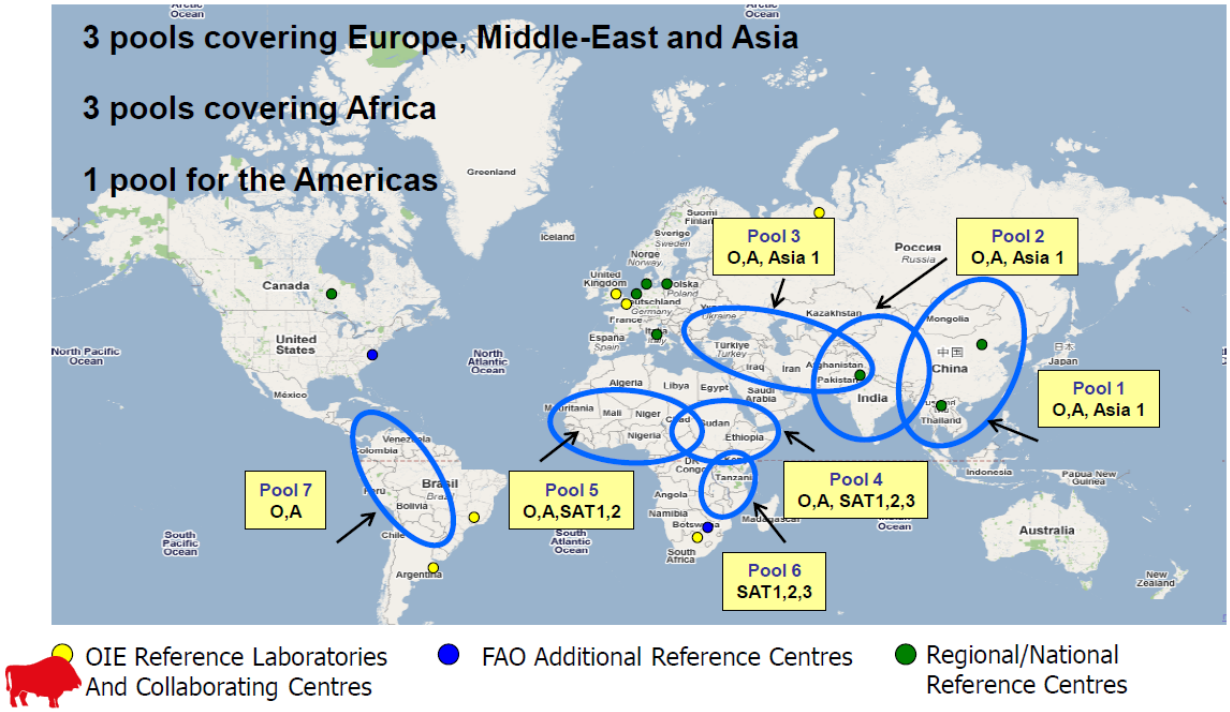

Figure B-1. Global distribution of FMD virus (Paton et al., 2009).
http://www.wrlfmd.org/fmd_presentations/fmd_presentations.htm

Human Disease (Zoonotic Potential)

FMDv is not considered a zoonotic disease. Cases of human disease are extremely rare and require direct contact and handling of infected livestock, infected laboratory tissues/equipment, or ingestion of infected milk. When infection has occurred, symptoms are usually temporary and mild (fever, vesicles on the hands, feet, or in the mouth). In contrast, Hand, Foot, and Mouth disease (HFMD) in humans is an unrelated viral disease caused by a different virus that primarily affects infants and children. The human disease is often confused with FMDv of livestock. Humans play a significant role in the passive transfer of FMDv from infected animals or contaminated surfaces to susceptible animals, and they may even passively carry the virus in the respiratory tract for a day or more (Alexandersen and Mowat, 2005).

Resistance to Chemical and Physical Agents

Background on Stability of FMD in Milk Products

Blackwell and Hyde, (1976) demonstrated that FMDv replicates in the mammary gland and proposed that the virus is incorporated into casein micelles and fat droplets which protect the virus from environmental inactivants such as heat (Blackwell and Hyde, 1976). FMDv exhibits enhanced stability in milk and dairy products compared to FMDv in laboratory suspensions, which suggests that the milk fat globule (MFG) and casein protect the virus. The factors in milk that protect the virus from heat include the high protein content, solid matter, and fat content.

These factors also affect the efficacy of disinfection of FMDv in milk (Dr. Nick Juleff, personal communication, 2012). Ferris et al., (1990) showed that the addition of sucrose and lactalbumin, or skimmed milk powder, stabilized FMDv effectively during and after freeze-drying (Ferris et al., 1990).

The virus survives in dairy products of diverse composition including casein (protein), butter and butter oil (lipid), and cheese (a combination of lipid and protein) (Blackwell et al., 1983). The virus was not detected in acid whey (pH 4.6), a by-product of casein manufacture. However, FMDv survived in desiccated and wet casein slurries (pH 4.6) and in sweet whey (pH 6.2) suggesting that the composition of casein, as well as that of respective whey samples, was a factor in virus survival (Blackwell et al., 1983). Using both physical and chemical methods to disrupt milk components collected from infectious cows, Blackwell et al., (1983) observed that the viral titers in buttermilk (after churning) were higher than those of the original cream or butter samples from the infected cows (Blackwell et al., 1983). Entrapped FMDv was released from milk fat globules after apparent chemical disruption of the membrane with an anionic detergent sodium dodecyl sulfate (SDS), suggesting an association of virus with milk fat globules. In skim milk samples treated with SDS, there was a consistent increase in the viral titer by 1 log, suggesting that the virus was released through removal of protein material and dissociation of casein aggregates. This effect was not achieved with chelating agents or trypsin.

Laboratory Studies on pH and Temperature Inactivation of Virus in Tissue Suspension

In early FMDv studies under laboratory conditions with virus grown in tissue cultures or virus added to a tissue suspension, the virus was shown to be readily inactivated by physical agents such as heat, extremes of pH, and desiccation. In non-milk systems, FMDv was readily inactivated by a number of disinfectants at pH extremes. Organic acids at a pH of 6.5 and below completely inactivated the virus in fluid suspensions and on inanimate surfaces (Blackwell et al., 1983); Baltimore, 1969; Sellers, 1968). Most strains of FMDv were stable within the pH range of 7.0 to 7.5, especially at lower temperatures (Bachrach et al., 1957a). At pH values outside of this range, the strains became increasingly unstable and where rapidly inactivated at pH < 4 and pH > 11 (Alexandersen et al., 2003c; Bachrach, 1968; Bachrach et al., 1957a).

The pH dependent inactivation of FMDv is biphasic in nature with a small fraction of virus surviving for longer periods (Alexandersen, 2005). Ninety percent of the virus population in suspension was inactivated within 1 second and 1 minute respectively at pH values of 5 and 6. A small fraction (one in one-millionth) of the virus population that remained had a much lower first order inactivation rate compared to the bulk titer of virus. This residual virus was very stable to further inactivation (Bachrach, 1968). **Table B-1** and **Table B-2** present the effect of temperature and pH on time for 1 $\log_{10}$ reduction (90 percent inactivation) of virus in laboratory suspension (Bachrach, 1968; Bachrach et al., 1957a).

Temperatures above 50 °C inactivate most of the virus present, but a small proportion of virus particles will likely persist (one millionth of the initial concentration) and the remaining population will be very resistant to heat treatment (Bachrach et al., 1957a; Hyslop, 1965). Organic materials, such as blood and feces, provide some protection against heat inactivation of FMDv (Alexandersen, 2005). The rate of inactivation of FMDv is dependent on many parameters, including the content of organic material and the ionic strength of the mixture. The effect of lowering the pH is dependent on the actual pH that is achieved and the duration of the treatment (Alexandersen, 2005). In milk products, in particular, the exact quantitative effect of lowering the pH on virus infectivity may be difficult to assess (Alexandersen, 2005).

Table B-1. Effect of combination of temperatures and time for 90 percent FMDv inactivation, in suspension at pH 7.5 (from Bachrach 1957, 1968).

Temperature (° Fahrenheit)	Temperature (° Celsius)	Inactivation Time (90%)
142°F	61°C	3 seconds
131°F	55°C	20 seconds
120°F	49°C	1 hour
109°F	43°C	7 hours
99°F	37°C	21 hours
68°F	20°C	11 days
39°F	4°C	18 weeks

Table B-2. Effect of pH on time for 90 percent FMDv inactivation in suspension at 4 °C (from Bachrach 1957, 1968).

Effect of pH on Inactivation Time	
pH	**Inactivation Time (90%)**
10	14 hours
9	1 week
8	3 weeks
7 – 7.5	> 5 weeks
6.5	14 hours
6	1 minute
5	1 second

Studies on pH and Temperature Inactivation of Virus in Milk Systems

The pH of FMDv infected milk (pH 7.1) has been noted to be higher than milk from uninfected cows (pH 6.7) and lies within the pH range where FMDv is most stable (Hyde et al., 1975; Tomasula et al., 2007). FMDv in suspension is most stable between a pH of 7 and 7.5 when subjected to heating (Bachrach, 1961; Bachrach et al., 1957b). Thermal inactivation kinetics of FMDv in milk indicate that milk with pH > 7 may be inactivated more slowly by heat than milk

with a pH < 7 (Alexandersen, 2005). Sellers (1968) reported a $10^{4.5}$ to > 10^{5} log reduction of FMDv titer after lowering the pH to 6 for 30 minutes at 4°C, unless the material contained milk (40 percent), for which he noted only a $10^{2.2}$ log reduction in titer (Alexandersen, 2005).

Studies have documented the thermal and pH stability of FMDv in milk (Blackwell and Hyde (1976); Hyde et al. (1975) and DeLeeuw and Bekkum, 1979). The stability of the virus is greater when FMDv is present as a result of infection of the animal rather than when the virus is added to milk (DeLeeuw and Bekkum, 1979). This difference in stability was attributed to the possible association between virus and the milk components. Primarily infected milk refers to infectious milk produced by direct inoculation of the virus into the udder and concurrent intravenous injection into the jugular vein of the cow. Virus in milk produced by this route has been shown to have greater resistance to heat inactivation compared to milk in which virus was added, because of the protective effect of the milk constituents (DeLeeuw and Bekkum, 1979). For FMDv infectious milk, additional time may be required above the normal holding times (for standard pasteurization times and temperatures) for heat to penetrate the milk fat globule membrane, liquefy the fat, and inactivate the virus encapsulated within the fat globules (Tomasula et al., 2007).

Thermal Inactivation via Pasteurization Simulation Studies

Laboratory studies that simulate HTST pasteurization have shown that FMDv is not completely inactivated at the minimum legal pasteurization requirements of 72 °C / 15 seconds (Hyde et al., 1975; Tomasula et al., 2007). The heat resistance of FMDv serotype A present in naturally infected whole milk has been evaluated (Hyde et al., 1975; and Blackwell and Hyde (1976). This milk is produced experimentally by inoculating cows with FMDv, or placing susceptible cows in contact with FMD inoculated animals. Both studies used a laboratory batch processing technique to simulate HTST pasteurization by heating milk to 72 °C / 15 seconds, the PMO legal time and temperature conditions for pasteurization (FDA US Department of Human Health Services, 2009). Cell culture techniques and steer inoculation were used to assay for virus post-treatment. Cell culture techniques are useful for enumerating high concentrations of viral plaques, but it is not a sensitive technique when virus titers are low. Steer inoculation was used because it is the most sensitive method known to detect infectious FMDv present at low concentrations. Milk samples that were negative for virus isolation in cell culture were inoculated into a single steer using a 2 ml intradermal (ID) injection into 20 sites in the tongue and 35 ml inoculation intramuscularly (IM) into 4 sites in the gluteal muscles (Tomasula et al., 2007). The study results are presented below.

Primarily infected milk (collected 1-day post-inoculation) was used to study the effect of pasteurization using temperatures of 72 °C and 80 °C and holding times of 15 to 17 seconds (Hyde et al., 1975). The results indicate:

- Initial milk concentrations ranged from 6.7 to 7.5 $\log_{10}$ PFU/ml, with a mean pH of 7.15.

- Virus titers, after pasteurization, ranged from 1.6 to 3.0 $\log_{10}$ PFU/ml (72 °C) and 0.91 to 3.0 $\log_{10}$ PFU/ml (80 °C).
- Reduction in virus titer ranged from 3.7 to 5.5 $\log_{10}$ (72°C) and 4.7 to 5.99 $\log_{10}$ (80 °C).
- All samples were negative using cell culture techniques, but were positive by inoculation into susceptible cattle.

Infected milk pasteurized and evaporated to 50 percent of the original volume was also evaluated by heating at 65 °C for appropriately 65 minutes (Hyde et al., 1975). These samples were negative for virus by cell culture assay, but when similar samples were inoculated into steers, vesicular lesions were produced within 2 days.

Batch processing pasteurization of primarily infected (udder and IV inoculation) whole and skim milk in 3 cows was evaluated (Blackwell and Hyde (1976). The samples were collected 1-day post-inoculation and were heated at 72 °C for 7 different time intervals (0.25, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 minutes). One sample of whole and skim milk from each cow was evaluated at each time step. In a second experiment, milk samples were collected 1 to 4 days after inoculation, and separated into whole, skim, cream, and pelleted fractions. Virus titers were consistently highest in the cream fraction. Cream fraction samples were inoculated into steers after heating at 72 °C and 93 °C for 0.25 minutes. The heat inactivation study results were:

Whole Milk Heated at 72 °C for 7 Different Time Steps

- Initial FMDv milk titers were 4.95, 5.3, and 6.4 $\log_{10}$ PFU/ml for each of the 3 cow samples.
- Up to a 6 $\log_{10}$ reduction in titer was observed after heat treatment. All samples titers were less than 0.4 $\log_{10}$ PFU/ml in cell culture after heat treatment, with 3 exceptions noted below.
- FMDv was detected in cell culture assays at concentrations of 0.5 $\log_{10}$ PFU/ml after heating for 0.5 and 1 minute in samples from cow #1 and at 4 minutes (concentration of 1.2 $\log_{10}$ PFU/ml) in one sample from cow #2.
- Steer inoculation studies indicated that FMDv survived in all of the heat-treated whole milk samples from all 3 cows at each time step (0.5 to 5 minutes) as evidenced by development of clinical disease.
- In another evaluation, FMDv also survived in whole milk after heating at 85 °C for 0.25 minutes.

Skim Milk Heated at 72 °C for 7 Different Time Steps

- Initial FMDv milk titers were 6.3, 4.6, 4.7 $\log_{10}$ PFU/ml for each of the 3 cow samples.

- After heating at each time step, all samples titers contained fewer than 0.4 $\log_{10}$ PFU/ml with one exception.
- FMDv was detected in cell culture assay at 1.0 minute (concentration of 0.47 $\log_{10}$ PFU/ml) in cow #1.
- Steer inoculation studies indicated that FMDv survived in skim milk from all 3 cow samples heated at 72 °C for 1 minute.
- The milk sample from cow #3 was infective for steers when heated at 72 °C for 2 minutes.

Cream Heated at 72 °C and 93 °C for 0.25 Minutes

- Initial pre-treatment titers were 1.8, 5.6, and 6.3 $\log_{10}$ PFU/ml.
- After heat treatment at 72 °C, samples titers were < 0.4, 0.51, and 1.0 $\log_{10}$ PFU/ml.
- After heat treatment at 93°C, titer for all samples were < 0.4 $\log_{10}$ PFU/ml.
- All cream samples from the 3 cows heated at 72 °C and 93 °C for 0.25 minutes were infectious to the steers.

Milk after Evaporation to 50 Percent Volume

- FMD survived in whole milk heated at 72 °C for 3 minutes and then evaporated.
- The virus survived in 1 of 3 skim milk samples heated at 72 °C for 0.25 minutes and then evaporated.
- Virus survived in whole milk after heating at 72 °C for 3 minutes and then evaporated.
- Virus survived in cream after heating at 93 °C for 0.25 minutes.

Conclusion of the Study from the Authors

FMDv survives in skim milk, cream, and pelleted cellular debris components of milk obtained from FMDv-infected cows after pasteurization at 72 °C for 0.25 minutes (15 seconds). Virus was recovered from whole milk of infected cows after the milk was heated at 72 °C for 5 minutes (longest time tested) and from skim milk after heating at the same temperature for 2 minutes. Evaporation of whole milk samples after heating at 72 °C for 3 minutes did not inactivate the virus, but evaporation of infected skim milk samples heated at 72 °C for 0.5 minutes did inactivate the virus. The temperatures used in this study to evaluate inactivation in cream (93 °C for 0.25 minutes) are not adequate for the inactivation of FMDv and points out the protection that cream would confer to virus present in dairy products prepared from whole milk from infected cows. The fact that FMDv survives the evaporation of whole milk heated for expanded exposure periods, but not that of skim milk samples, suggests that butterfat in cream of whole milk confers an even higher degree of protection during heating than does the protein in skim milk. Blackwell and Hyde (1976) concluded that pasteurization was less effective for elimination

of virus in whole milk because the milk fat and casein proteins offered some protection to the virus (Blackwell and Hyde (1976).

Walker et al, (1984) constructed a thermal death time curve for FMDv using primarily infected milk Walker et al, (1984). Whole and skim milk samples were exposed to various temperatures (80 °C to 148 °C) and time (2.5 to 27 minutes) ranges and then tested for viral infectivity by inoculation into steers. The average pre-treatment milk titer using 53 lots of milk was 5.9 $\log_{10}$ PFU/ml. The thermal death time curve generated indicated that in order to inactivate the virus, times of over 20 minutes are necessary at low temperature (100 °C), while 2.5 seconds is sufficient at a temperature of 148 °C. No difference in viral inactivation was noted in skim and whole milk. Tomasula et al., (2007) stated that if the graph data from the Walker et al., (1984) are extrapolated to the 72 °C point, the corresponding time necessary for virus inactivation is approximately 230 minutes (Tomasula et al., 2007; Walker et al., 1984). It is unclear from the results whether the elevated temperatures alone were responsible for inactivation of the virus or whether the heating of milk under continuous flow conditions contributed to inactivation of the virus (Tomasula et al., 2007).

Tomasula et al. (2007) evaluated whether laboratory simulation of HTST pasteurization conducted in a continuous flow pasteurizer would be more effective than laboratory batch pasteurization methods (used by Blackwell and Hyde, 1976) in evaluating the effect of pasteurization on FMDv infectivity in naturally infected milk (Tomasula et al., 2007). Batch methods may have problems with evaporative cooling or splashing of milk, which can underestimate the effectiveness of the pasteurization process. In this study, samples of primarily infected milk containing FMDv serotype 01/UK at levels up to 4 $\log_{10}$ PFU/ml were pasteurized at temperatures ranging from 72 to 95 °C using holding times of either 18.6 or 36 seconds. The results indicate:

- Titer concentrations prior to pasteurization ranged from 1.92×10^2 to 9.25×10^3 PFU/ml.
- Pasteurization decreased virus infectivity by 4 $\log_{10}$ to undetectable levels in tissue culture.
- Whole milk samples pasteurized at 72 °C and 18.6 seconds, although negative by virus isolation in tissue culture, were positive by steer inoculation tests (2 samples evaluated).
- In milk containing the lowest titer of virus 1.92×10^2 PFU/ml, there was adequate residual viral infectivity remaining to cause disease in inoculated steers.
- Whole milk samples pasteurized at 80 °C, and held for either 18.6 or 36 seconds, were negative by cell culture as well.

This study, and that of Blackwell and Hyde (1976), indicates that FMDv encapsulated by milk fat is resistant to pasteurization (Blackwell and Hyde, 1976). Although they were able to detect residual infectivity in milk by directly inoculating relatively large volumes of milk intradermally

and IM in to steers, the ability of this milk to infect by oral or other routes is unlikely (Tomasula et al., 2007). The authors concluded that although the HTST pasteurization did not completely inactivate viral infectivity in whole and skim milk, it greatly reduced the risk of natural transmission of FMDv to animals by milk (Tomasula et al., 2007).

Heat Inactivation

Dekker (1998) evaluated the heat inactivation of 4 types of FMDv (A_{10} Holland, O_1 BFS, C_1 Detmold and Asia-1 SAU32/92) by air drying virus onto coverslips and subjecting them to heat treatment at 37 °C, 50 °C and 80 °C (Dekker, 1998). Suspensions of each of the 4 types were treated similarly. The reduction in titer due to drying varied greatly between the different types of FMDv. Type A seemed to be more stable than the other 3 serotypes. Heat inactivation of coverslips at 37 °C for 14 days, 50 °C for 2 days or 80 °C for 1 hour was not sufficient to completely inactivate 3 of the 4 types of FMDv. No infectious virus was detected in the virus suspensions heated at 37 °C for 7 days, 50 °C for 2 days, or 80 °C for 3.75 minutes.

The results demonstrated that dry heat is an inadequate method for inactivation of air-dried FMDv. The experiments confirm the observations of Bachrach et al., (1957a) that FMDv in suspension can be inactivated by dry heat at 50 °C in two days. On the other hand, FMDv that was air-dried on coverslips was found to be highly resistant to heat treatment. The author(s) concluded that the failure of dry heat to adequately inactivate air-dried FMDv is probably due to the high concentration of protein remaining after the virus has been dried.

Ferris et al., (1990) showed that the addition of sucrose and lactalbumin, or skimmed milk powder, stabilized FMDv efficiently during and after freeze-drying (Ferris et al., 1990). Using a 5 percent skimmed milk powder as a stabilizer, they observed a titer reduction of only 0.4 to 1.1 $\log_{10}$ for virus held at 37 °C for 7 days. This reduction in titer is comparable to the results of the Dekker study (Dekker, 1998).

Chemical Inactivation

Sigurdson et al., (2004) stated that in some of the early studies on FMDv it was found that the virus can be inactivated by very high or very low pH (Sigurdson et al., 2004). While this may be effective against the virus in a very clean environment, the presence of organic load will drastically affect the pH and thus efficacy. Infectious agents vary in their resistance to disinfectants and FMDv is among the most resistant of infectious agents (Sigurdson et al., 2004).

Krug et al., (2011a) tested the efficacy of disinfectants against three animal disease agents (FMDv, African Swine virus, and Classical Swine Fever virus) dried on steel and plastic surfaces (fomites). The study was designed to obtain basic information regarding the “disinfectability” of the virus. The approach was based on drying high-titer stocks of virus in low concentrations of organic material. Most of the published disinfection data has been generated from liquid suspension studies, which provides basic information about viral sensitivity to various chemicals.

These studies have served as the basis for the use of disinfectants in the field, yet it is known that disinfectant efficacy is reduced when applied to dried viruses (Sattar and Springthorpe, 1996).

The authors used higher titer virus stocks (minimum concentration of 4.8 $\log_{10}$ $CCID_{50}$ (cell culture infective dose equivalent to $TCID_{50}$) diluted in PBS and then dried on stainless steel and plastic surfaces. The initial drying process resulted in approximately a 2-$\log_{10}$ reduction in infectivity of FMDv on either steel or plastic surfaces. Various concentrations of disinfectant were applied to the dried virus preparations for 10-minute contact time and then the titer of remaining virus was determined after neutralization. Sodium hypochlorite (1,000 ppm) completely disinfected FMDv on both surfaces in 4 minutes. Citric acid was capable of reducing FMDv by greater than 4-$\log_{10}$ units on both plastic and steel surfaces. **Table B-3** presents the chemical disinfectant and the associated titer reduction.

Table B-3. Log_{10} reduction of FMDv by chemical disinfectants on nonporous surfaces (Krug et al., 2011a).

	Disinfectant			
Surface	**500ppm Hypochlorite**	**1,000ppm Hypochlorite**	**0.5% Citric Acid ***	**1.0% Citric Acid**
Steel	2.63 ± 0.53	5.55 ± 0.47	4.7 ± 0.88	5.0 ± 0.35
Plastic	2.38 ± 0.18	5.6 ± 0.8	4.13 ±0.72	5.1 ± 0.49

Log_{10} reduction by indicated disinfectant (mean ± SD)
* = disinfection did not reduce virus to undetectable levels

The authors were unable to achieve complete disinfection of dried FMDv with a 0.5 percent citric acid solution, indicating that the 0.2 percent citric acid recommended by OIE may not always be sufficient to completely disinfect dried FMDv in the field (Krug et al., 2011a). Research at Plum Island indicated that 0.2 percent citric acid was not effective at complete inactivation of very high-titer dried virus, but it was able to disinfect close to 4 logs of dried FMDv on nonporous surfaces (Krug, 2012).

Sodium carbonate (soda ash) has commonly been used as a disinfectant for transport vehicles moving between FMDv endemic and non-endemic countries. It is not registered by EPA as an FMDv disinfectant, but has been given an exemption by the USDA (Krug et al., 2011a). Sodium carbonate (4 percent) produced a 4-log reduction in FMDv titers, but the treatment did not completely disinfect dried inoculums.

Krug et al., (2011b) also tested the efficacy of disinfectants on a porous surface (Krug et al., 2011). Drying of FMDv onto birch coupons (test surfaces) resulted in a 1.8 $\log_{10}$ reduction in virus titer due to drying alone. Citric acid (2 percent) was able to reduce FMDv by greater than 4 $\log_{10}$ at 20 minutes; however, 1,000 ppm sodium hypochlorite was not able to do so by 30 minutes. Both disinfectants rapidly inactivated the virus bound to the outer surface of the wood in the first 10 minutes, but the hypochlorite was either inhibited or consumed by the wood itself

in the next 20 minutes. Citric acid continued the inactivation trend and, by 25 minutes, FMDv was reduced to below the limit of detection. Evaluation of 1,000 and 2,000 ppm hypochlorite showed minor efficacy differences, as treatment with the higher concentration did not result in a 4-log reduction in virus; 2,500 ppm sodium hypochlorite was not more effective at disinfecting birch-dried FMDv (Krug et al., 2011b). **Table B-4** presents the log reduction in FMDv for each disinfectant tested.

Table B-4. Log_{10} reduction of FMDv after disinfection on birch surfaces.

	Disinfectant		
	2% Citric Acid*	**Sodium Hypochlorite 1,000 ppm**	**Sodium Hypochlorite 2,000 ppm**
Titer reduction (Log_{10}) after 30 minutes	5.22 ± 0.78	2.95 ± 0.57	3.77 ± 0.44
Number of positive coupons/total number of coupons inoculated.	4/20	11/11	11/11

*Citric acid contact time was 25 minutes

The difference between the effectiveness of the 2 percent citric acid and sodium hypochlorite may be due to the consumption of the hypochlorite by the wood itself, as the activity of hypochlorite can be inhibited in the presence of organic material (Krug et al., 2011b). Another possibility is the diminished ability of hypochlorite solutions to penetrate wood compared to acidic solutions. In summary, disinfection of FMDv fomites is dependent on the type of material in the sample. If a significant amount of organic material is present, then bleach is not a good choice as it will be inactivated by the organic material. Acids would then be the best choice because they have good efficacy in the presence of organic material. Another variable to consider is the surface porosity. Krug et al., (2011b) observed that the citric acid concentration needed to be increased to 2 percent to get effective 4 log_{10} inactivation of FMDv dried on wood surfaces. For FMDv disinfection, all surfaces must be cleaned first—which will remove much of the virus—however, the problem becomes dealing with contaminated effluent. Bleach will not be effective in the presence of milk proteins, even after a prior surface cleaning (Krug, 2012).

Environmental Persistence

FMDv is one of the most contagious infectious agents known due to its rapid replication cycle and its stability in the environment. The non-enveloped virus maintains infectivity in the environment long after drying, thus fomites are a major mechanism of virus spread (Krug et al., 2011a). Survival is dependent on the pH, temperature, humidity, and initial concentration of the virus. The reported survival times of virus in environmental media are listed below (Alexandersen et al., 2003c):

- up to 20 weeks on hay or straw
- up to 4 weeks on cows' hair at 18 to 20 °C

- up to 14 days in dry feces
- up to 39 days in urine
- up to 6 months in manure slurry in the winter
- up to 14 days in dry feces and up to 39 days in urine
- 3 days on soil in summer and 28 days in autumn
- 2 to 3 months in bovine feces or slurry (Haas et al., 1995; Parker, 1971)

Such observations have generally been made in countries with a temperate climate; these times would be expected to be much shorter in countries with hot climates (Geering and Lubroth, 2002). The important criterion is whether, at the time of animal exposure, there is sufficient residual infectivity in the material or environment to initiate infection. The length of time virus will survive in the environment is difficult to predict because the data from different studies are not comparable due to differences in experimental design and assay methods used. Restocking after an outbreak has to be done with care, only after thorough disinfection of the premises and preferably with the initial introduction and monitoring of sentinel animals (Alexandersen et al., 2003c).

Environmental factors that favor survival of virus are moist conditions, a neutral pH, and low temperature. Virus excreted during the preclinical and acute clinical phases can survive in the environment for weeks or even months. Survival of the virus in the environment will depend on the nature of the material (desquamated epithelium, secretions, and excretions), initial concentration of virus in the material, strain of virus, humidity, pH, and temperature and therefore will be highly variable under field conditions. Residual virus may be remarkably resistant, especially in the presence of high concentrations of organic material (Alexandersen et al., 2003c). Virus survival will also depend on the type of material contaminated such as manure, fodder, bedding, footwear, clothing, equipment, vehicles, and other fomites.

Table B-5 presents a list of fomites that were ranked as moderate or high hazards and their survival time in the environment. While there have been isolated reports of survival of virus for extended periods (on hay for at least 200 days, in fecal slurry for 6 months (Hyslop, 1970), few quantitative data are available on the persistence of FMDv in the environment (Cottral, 1969; Donaldson, 1997; Sanson, 1994).

Table B-5. Fomites ranked as moderate, or high hazards, and the estimated survival times of FMDv under different conditions (USDA:APHIS:VS:CEAH, 2007).

Fomite	Conditions	Virus Survival Time
Shoes or boots	Summer	9 weeks
	Winter	14 weeks
Clothing	–	100 days
	Summer	9 weeks
	Winter	14 weeks
Bedding (straw, wood shavings)	–	4 weeks
Soil	Summer	3-7 days
	Autumn	4 weeks
	Winter	21 weeks

The important criterion to determine the risks associated with environmental contamination is whether, at the time of exposure, there is sufficient residual infectivity in the material or environment to initiate infection. Large amounts of virus are excreted in animal secretions and excretions including vesicular fluid, desquamated vesicular epithelium, and saliva and become mixed with manure and cause environmental contamination. The excretion of virus in feces is expected to be at levels lower than those associated with vesicular fluids, nasal, and oral secretions (Alexandersen et al., 2003c; Burrows, 1968; Garland, 1974; Parker, 1971).

Concentrations in cow nasal secretions and saliva range from 5.5 $\log_{10}$ to 8.8 $\log_{10}$ $TCID_{50}$/ml in research studies (Alexandersen et al., 2003c). Kitching (2002) states that urine may contain FMDv levels of 4.6 $\log_{10}$ $TCID_{50}$/ml and feces 5.0 $\log_{10}$ $TCID_{50}$/g. These data does not indicate the disease stage when these levels could occur. The maximum infectivity titers for manure in cattle experimentally infected with FMD are shown in **Table B-6**.

Table B-6. Virus titers in manure from experimental studies (Alexandersen et al., 2003c).

Virus Strain	Maximum Recorded Viral Titer	Theoretical Total Viral Content*	Reference
O Canefa-2	4.1 $\log_{10}$ $TCID_{50}$/g	8.7 $\log_{10}$	Sutmoller and McVicar 1973
O BFS 1860	2.0 $\log_{10}$ PFU/g	6.7 $\log_{10}$	Sellers et al., 1969
O Swiss 1/66	3.0 $\log_{10}$ $TCID_{50}$/g	7.7 $\log_{10}$	Garland 1974
A 119	2.0 $\log_{10}$ $TCID_{50}$/g	6.7 $\log_{10}$	Garland 1974
C Noville	3.3 $\log_{10}$ $TCID_{50}$/g	8.0 $\log_{10}$	Garland 1974

*Theoretical calculation assuming that the daily output is contaminated to the maximum detected level of infectivity. The theoretical value for the total daily amount of viral infectivity is based on the amount of each fluid normally produced per day and on the assumption that the entire daily output is contaminated to the maximum level cited.

The experimental values listed above should be considered relative values as they were obtained using different strains, assay systems, and study methods. The values are useful as indicators of the high infectivity level obtained by manure alone and the consequent massive environmental contamination. All secretions and excretions are important in mechanical spread; especially when people become contaminated by excretions and secretions such as milk, vesicular fluid, saliva, urine, or feces and then handle other animals. Other mechanical means of spread include the use of animal transport vehicles which have not been cleaned and disinfected (Alexandersen et al., 2003c)

Diagnosis

Diagnosis of FMDv is initially based on appearance of clinical signs; definitive diagnosis requires laboratory confirmation. Collection of specimens is essential for confirmation and is dependent on the choice of the diagnostic method. Specimens include vesicular fluid, vesicular epithelium, swabs of erosive lesions, oral swabs, and acute and convalescent serum samples (United States Animal Health Association, 2008). A variety of laboratory tests may be carried out on diagnostic samples. The laboratory tests currently available at the Foreign Animal Disease Diagnostic Laboratory (FADDL) at Plum Island, NY for the diagnosis of FMDv are shown in **Table B-7**. At the time of this risk assessment, no rapid or on-farm test approved for use in the United States by the USDA was available.

Table B-7. Laboratory tests currently available at FADDL for the diagnosis of FMDv.

Test	Specimen	Test detects	Time to Result
Real-time RT-PCR	tissues, cell cultures, etc.	viral RNA	2 – 3 hours
Antigen ELISA	vesicular fluids, epithelial tissues, cell cultures, etc.	antigen and serotype	5 – 6 hours
Virus isolation	épithélial tissues, vesicular fluid, swabs, etc.	virus	3 – 7 days
AGID	serum	antibody to VIAA	24 hours
PrioCHECK® FMDv-NS	serum	antibody to NSP 3ABC	20 hours
VNT	serum	antibody to SP and serotype identification	2 – 3 days

Appendix C. Simulation Model for Estimating the FMDv Concentrations

This appendix provides technical details and mathematical equations used in the disease spread simulation model (SLIR) to determine the concentration of infectious virus that could be present in the bulk tank on an infected but undetected farm.

Stochastic Disease Transmission Module

The objective of this module is to simulate the spread of FMD virus within a herd and estimate the number of cows in various disease states at different periods. The disease states included in the module are susceptible (S), latent (L), preclinically infectious (PI), clinically infectious (CI) and recovered (R) (Perez et al., 2004). The module updates the number of cows in the disease states at specific time steps (e.g., every 6 hours). The uncertainties in the input variables, as well as the inherent variability associated with the course of FMD infection in cattle and disease spread within the herd, were considered in the model.

Assumptions and Notation

We have the following notation for this section:

N – The number of cows in the herd

i – index of individual cows on the farm i $\in 1, \ldots, N$

t – index of time periods $t \in 1, \ldots, t$

$\widehat{\boldsymbol{P}}\boldsymbol{(t)}$ – Set of indices of cows that are in a susceptible state in time period t

$\widehat{\boldsymbol{L}}(\boldsymbol{t})$ – Set of indices of cows that are in a latent state in time period t

$\widehat{\boldsymbol{SI}}(\boldsymbol{t})$ – Set of indices of cows that are in a preclinically infectious state in time period t

$\widehat{\boldsymbol{CI}}(\boldsymbol{t})$ – Set of indices of cows that are in a clinically infectious state in time period t

$\widehat{\boldsymbol{R}}(\boldsymbol{t})$ – Set of indices of cows that are in an immune state in time period t

P_t – Probability that a cow that is susceptible in period t becomes infected with FMD by time period t+1

$N_i(t)$ – Number of infectious cows in time period t

K – Adequate contact rate, the expected number of contacts that a cow has with other cows in a time period that is adequate to transmit FMD infection

$I_{t,t+1}^{new}$ – Number of newly infected cows between time period t and t+1

$N_s(t)$ – The number of susceptible cows in time period *t*

$\tau^L(i)$ – A model variable denoting the length of the latently infected period for a cow in a specific simulation iteration. This parameter was simulated using an exponential distribution as detailed in section 8.3

$\tau^{SI}(i)$ – A model variable denoting the length of the preclinically infectious period for a cow in a specific simulation iteration. This parameter was simulated using a lognormal distribution as detailed in section 8.3

$\tau^{CI}(i)$ – A model variable denoting the length of the clinically infectious period for a cow in a specific simulation iteration. This parameter was simulated using a gamma distribution as detailed in section 8.3

$T^L(i)$ – A model variable denoting the simulation time at which a cow *i* entered into latently infected state

$T^{SI}(i)$ – A model variable denoting the simulation time at which a cow *i* entered into pre clinically infectious state

$T^{CI}(i)$ – A model variable denoting the simulation time at which a cow *i* entered into clinically infectious state

λ – The number of hours represented by each time period *t* of the simulation model — λ was set at 6 hours in our simulations

The main assumptions associated with this module are listed below:

- The cows that are in susceptible state in a time period all have an identical probability of becoming infected by the next period (i.e., differences in transmission due to grouping of cows in pens are not considered).
- Preclinically infectious or clinically infectious cows are both equally infective with respect to transmitting FMD, if they have an adequate contact with a susceptible cow.
- The number of adequate contacts per cow in a period follows the Poisson distribution.
- The variability in adequate contact rate due to differences in density of cows (number of cows/unit area) among different dairy operations is not considered (i.e., the transmission is modeled as being frequency dependent).
- The clinically immune state is not considered in the model.

The Transmission Equation

The transmission equation estimates the number of susceptible cows that become newly infected with FMD in each time period. The transmission equation is based on calculation of the

probability that a susceptible cow has an adequate contact with at least one infected cow in a time period. The variables considered in the equation include the adequate contact rate, the number of infectious cows, the number of susceptible cows, and the total number of cows in the herd. In general, a higher adequate contact rate, or higher proportion of infectious cows, will lead to increased transmission. We use the transmission equation derived in Dietz and Schenzle (1985) as shown in Equation 1 (Dietz and Schenzle, 1985). This transmission equation assumes that the number of adequate contacts each cow has in a period is Poisson distributed with a mean (*k*). A Poisson process indicates a continuous and constant opportunity for an event to occur.

$$P_t = 1 - e^{-\frac{k(N_i(t))}{N-1}} \qquad \text{(Appendix C EQ. 1)}$$

$$I_{t,t+1}^{new} \sim Binomial(S, P_t) \qquad \text{(Appendix C EQ. 2)}$$

Transition between Different Disease States

The transmission equation provides the basis for calculating the number of cows transitioning from susceptible to the latently infected state in one time period. In this section, we briefly describe the implementation details for transitions between other disease states. As stated earlier, the model updates the disease states of the cows in unit time steps (e.g., 6 hours). The transitions from latently infected to preclinically infectious, preclinically infectious to clinically infectious, and clinically infectious to recovered are based on keeping track of each individual cow's length of each disease state and timing of when a cow transitioned into a disease state. For instance, in the case of transitioning between the latent to preclinically infected state for a cow, the model first calculates the length of the latent period for the cow (τ^L) based on the latent time distribution. The model also keeps tracks of the time period when a cow transitioned into the latently infected state (T^L). The model transitions the cow from the latently infected to preclinically infected state in the first time period t where $t^*\lambda \geq \tau^L + T^L$. Other disease state transitions are performed in a similar manner. The main input parameters for this section of the model are the probability distributions of latent, preclinically infectious and clinically infectious time periods. The model can be run for a specified number of time periods and provides the estimates of the number of cows in various disease states. The main algorithmic steps associated with the various disease state transitions are provided below.

Algorithm steps for state transitions for a dairy herd in simulation iterations

Begin

For t = 1 to t_{max} {

$\widehat{\boldsymbol{R}}(\boldsymbol{t}+\mathbf{1}) = \widehat{\boldsymbol{R}}(\boldsymbol{t})$;

$\widehat{\boldsymbol{CI}}(\boldsymbol{t+1}) = \widehat{\boldsymbol{CI}}(\boldsymbol{t})$ **;**

$\widehat{\boldsymbol{SI}}(\boldsymbol{t+1}) = \widehat{\boldsymbol{SI}}(\boldsymbol{t})$ **;**

$\hat{\boldsymbol{L}}(\boldsymbol{t+1}) = \hat{\boldsymbol{L}}(\boldsymbol{t})$ **;**

$\hat{\boldsymbol{L}}(\boldsymbol{t+1}) = \hat{\boldsymbol{L}}(\boldsymbol{t})$ **;**

"Transitions between clinically infectious to immune state"

For each cow *i* ∈ $\widehat{\boldsymbol{CI}}(\boldsymbol{t+1})$ **{**

if $t^{*}\lambda \geq \tau^{CI}(i)+T^{CI}(i)$ then $\hat{\boldsymbol{R}}(\boldsymbol{t+1}) = \hat{\boldsymbol{R}}(\boldsymbol{t+1})$ U {i}, remove cow *i* from $\widehat{\boldsymbol{CI}}(\boldsymbol{t+1})$ **}** ;

"Transitions between preclinically infectious to clinically infectious state"

For each cow *i* ∈ $\widehat{\boldsymbol{SI}}(\boldsymbol{t+1})$ {

if $t^{*}\lambda \geq \tau^{SI}(i)+T^{SI}(i)$ then $\widehat{\boldsymbol{CI}}(\boldsymbol{t+1}) = \widehat{\boldsymbol{CI}}(\boldsymbol{t+1})$ U {i}, remove cow *i* from $\widehat{\boldsymbol{SI}}(\boldsymbol{t+1})$

$T^{CI}(i) = t + 1$ };

"Transitions between latent to preclinically infectious state."

For each cow *i* ∈ $\hat{\boldsymbol{L}}(\boldsymbol{t+1})$ {

if $t^{*}\lambda \geq \tau^{L}(i)+T^{L}(i)$ then $\widehat{\boldsymbol{SI}}(\boldsymbol{t+1}) = \widehat{\boldsymbol{SI}}(\boldsymbol{t+1})$ U {i}, remove cow *i* from $\hat{\boldsymbol{L}}(\boldsymbol{t+1})$

$T^{SI}(i) = t + 1$};

"Transitions between susceptible to latently infected state."

$P_t = 1 - e^{-\frac{k(N_i(t))}{N-1}}$;

$I_{t,t+1}^{new} \sim Binomial(N_s(t), P_t)$;

For each cow *i* among the first $I_{t,t+1}^{new}$ cows in $\widehat{\boldsymbol{S}}$***(t+1)*** **{**

$\hat{\boldsymbol{L}}(\boldsymbol{t+1}) = \hat{\boldsymbol{L}}(\boldsymbol{t+1})$U {i},

$T^{L}(i) = t + 1$};

Remove cow *i* from $\widehat{\boldsymbol{S}}$***(t+1)};***

}

END

Simulation of FMD Titer in a Bulk-Milk Tank

This module simulates the FMD titer in a bulk milk tank and updates it at each time step. The following factors are considered in estimating the FMD titer in a bulk-milk tank.

- The number of preclinically and clinically infectious cows in each time period, which is estimated by disease transmission module.
- The uncertainty and variability in the FMD titer of milk from preclinically and clinically infectious cows.
- The frequency at which the milk from the bulk tank is transported to the processing center.

The following simplifying assumptions were also made in implementing this module:

- Milk is added to the bulk-milk titer in each period.
- Milk production rate is a constant for all cows in the herd.

The module calculates the mean FMD milk titer (PFU/ml) of milk added to the bulk tank, based on the number of preclinically infectious and clinically infectious cows in that time step. The module then updates the FMD titer and amount of milk in the tank, while considering the volume and viral titer of the milk already present in the tank. The volume of milk present in the tanker is set to zero in each time period when there is milk transported to a processing center and the bulk tank is emptied. The dilution of FMDv titers in milk, due to mixing with milk collected on different days for smaller farms, is considered by the model. The model results of the milk titer in the bulk tank are presented in units of Log_{10} PFU/ml. We have the following additional notation for describing the simulation equations for estimating the milk titer:

Notation

$A_{in}(t)$ = Amount of milk added to the bulk tank in period *t* (ml)

$C_{in}(t)$ = Concentration of FMDv in milk added to the bulk tank in period *t* (PFU/ml)

$C_{msi}(t)$ = Average concentration of FMDv in milk produced by preclinically infectious cows in period *t*(PFU/ml)

$C_{mci}(t)$ = Average concentration of FMDv in milk produced by clinically infectious cows in period *t* (PFU/ml)

$A_{tank}(t)$ = Amount of milk added to the bulk tank in period *t* (ml)

$C_{tank}(t)$ = Concentration of FMDv in milk added to the bulk tank in period *t*(PFU/ml)

ρ = Milk production rate per-period per-cow (ml/period/cow)

Equations for Updating Milk Titer Between Periods (*t* and *t*+1)

$$A_{in}(t) = N\rho \quad \text{(Appendix C EQ. 3)}$$

$$C_{in}(t) = \frac{(\widehat{\boldsymbol{CI}}(\boldsymbol{t}) * \mathrm{C_{mci}}(\mathrm{t}) * \rho + \widehat{\boldsymbol{SI}}(\boldsymbol{t}) * \mathrm{C_{msi}}(\mathrm{t}) * \rho)}{A_{in}(t)} \quad \text{(Appendix C EQ. 4)}$$

$$A_{tank}(t+1) = A_{tank}(t) + A_{in}(t) \quad \text{(Appendix C EQ. 5)}$$

$$C_{tank}(t+1) = \frac{A_{tank}(t) * C_{tank}(t) + A_{in}(t) * C_{in}(t)}{A_{tank}(t+1)} \quad \text{(Appendix C EQ. 6)}$$

A_{tank} and C_{tank} are set to zero at a specific frequency to denote emptying of the bulk-milk tank.

Appendix D. Studies to Determine FMDv Milk Titer Data and Statistical Evaluation

Experimental Studies

The following information identifies four studies with experimental in-contact cow data (Blackwell, Reid, Borrows, and Orsel) and one study with outbreak data (Donaldson). The data are presented in **Table D-1**.

Blackwell et al., 1982

Concentrations of FMDv in Milk of Cows Infected under Simulated Field Conditions (Blackwell et al., 1982).

Susceptible lactating dairy cows were infected with FMD by exposure to infected pigs. Six cows were exposed to pigs infected with FMDv Brugge strain of O serotype, subtype 1 by inoculation into the footpad with 7 $\log_{10}$ PFU/ml. Six dairy cows were used; each recipient cow was placed in direct contact with two inoculated pigs. The recipient cows remained in contact with the infected pigs for the duration of the study of 15 days. Virus was detected in milk 1 to 7 days after exposure. Maximum infective titers in whole milk varied from 2.3 to 5.4 $\log_{10}$ pfu/ml and occurred 1 to 3 days after onset of virolactia. A second peak in the milk titer occurred on day 7 in cow 1 and on day 6 in cow 5. This is a high dose, direct contact study.

- Detected concentrations of virus in milk ranged from 0.82 to 5.5 log10 pfu/ml
- Preclinical stage concentration 0.83 pfu/ml to 4.7 log10 pfu/ml
- Clinical stage concentration 1.8 to 5.4 log 10 pfu/ml

Reid et al., 2004

Use of Automated RT-PCR to Detect FMDv in Milk (Reid et al., 2004) and Utility of Automated Real-time RT-PCR for the Detection of FMDv Excreted in Milk (Reid et al., 2006).

Four adult cows in mid-lactation were housed in pairs in two rooms. One cow of each pair was inoculated on day 0 by intra-dermolingual infection with 0.5 ml of FMD serotype Pan Asia type O UKG 34/2001 at a titer of $10^{5.9}$ $TCID_{50}$/ml/animal. Using the conversion of 1pfu = 1.4 $TCID_{50}$ values were converted to $\log_{10}$ pfu/ml. Results of this study showed:

- Concentrations in milk varied from 0.70 to 4.70 $TCID_{50}$/ml (0.55 to 4.55 $\log_{10}$ pfu/ml)
- Preclinical concentrations 0.95 to 1.2 $TCID_{50}$/ml (0.80 to 1.05 $\log_{10}$ pfu/ml)
- Clinical concentrations 0.45 to 4.2 $TCID_{50}$/ml (0.3 to 4.05 $\log_{10}$ pfu/ml)

Burrows et al., 1971

The Growth and Persistence of FMDv in the Bovine Mammary Gland (Burrows et al., 1971)

Donor animals were infected by intradermal inoculation of the tongue (1 steer), the coronary band (4 sheep), and the bulb of the heel (4 pigs). Donor animals were placed in 3 boxes and the recipient 4 dairy cattle were placed in the central area. The 2 groups were separated by at least 10 meters (aerosol exposure). Serotypes O-BSF 1860 and A22-Iraq24/64 were used. Milk production dropped by 30 to 50 percent commencing on the day that lesions were first seen. This study is of the airborne exposure route.

O1 exposure: concentrations in milk varied from 1.0 to 5.2 $\log_{10}$ pfu/ml.

- Preclinical concentrations 1.0 to 1.4 $\log_{10}$ pfu/ml
- Clinical concentration 2.4 to 5.2 $\log_{10}$ pfu/ml
- O1 cattle were not sampled after the 12th day of the experiment

A22 exposure concentrations in milk varied from 1.2 to 2.8 $\log_{10}$ pfu/ml.

- Preclinical concentrations: no detections
- Clinical concentrations: 1.2 to 2.8 $\log_{10}$pfu/ml
- The A22 cattle continued to excrete virus in the milk up to 19 days

K. Orsel et al., 2007

The Effect of Vaccination on FMDv Transmission among Dairy Cows (Orsel et al., 2007).

Infected cows were inoculated with FMD field isolate O/NET2001 in a dose of approximately 37,500 pfu (equal to 1,500 cattle –ID_{50}) by intranasal instillation. Five recipient cows were placed in contact with the five inoculated cows. Two replicate studies were conducted.

- Preclinical concentrations : reported as zero
- Clinical concentrations: 0 to 5.13 $\log_{10}$ pfu/ml
- Detection of clinical signs at 4 to 5 days

Donaldson et al., 1982

Use of Prediction Models to Forecast and Analyze Airborne Spread during the FMDv Outbreaks in Brittany, Jersey, and Isle of Wight in 1981 ***(Donaldson et al., 1982).***

Between March 4th and 26th, 1981 there were a series of 14 outbreaks of disease due to type O FMDv in France. The United Kingdom reported FMD type O in Jersey on March 19 and in the Isle of Wight on March 22. The outbreak took place at Hamstead farm. Two herds were kept on-

site, 32 milking cows, 38 young stock and 19 non-milking cows and heifers. The Lower Hamstead Farm had 80 beef cows. The owner noticed lameness in 1 of the 19 non-milking cows on March 20. On March 21, the number of lame animals had risen to 16 and the veterinarian was contacted. Depopulation was started on March 22. At the time of depopulation, the veterinary officer estimated that up to 8 cows (25 percent) of the milking herd were showing early signs of disease. The majority of this group had been buried and only 9 milking animals remained for investigation. None of these animals were found to have clinical lesions. Laboratory samples taken at this time indicated that disease was most advanced in the non-milking group, in the early stage of disease in the milking cows, and in the incubation period in some of the young stock. Milk samples were collected from the clinically normal cows, which suggested that they were nearing the end of the incubation period. Results of this study showed:

- Concentrations of virus in the milk of 6 clinically normal cows sampled
- Preclinical concentrations: 0.7, 6.6, 3.2, 1.5, 1.7, 1.1 $\log_{10}$ $TCID_{50}$/ml
- Bulk-milk sample: 2.2 $\log_{10}$ $TCID_{50}$/ml

Table D-1. Study data used in development of milk titer data.

Blackwell et al., 1982			***preclinical data determined by first detection in EP fluids***											
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
cow 1	0.00	1.00	*5.30*	**5.40**	5.00	4.40	4.40	4.60	0.00					
cow 2	0.00	0.00	0.00	*0.83*	0.83	**1.80**	2.50	0.00						
cow 3	0.00	0.00	0.00	0.00	*0.00*	0.00	0.00	4.70	5.50	3.40	2.60	0.00	0.00	0.00
cow 4	0.00	0.00	*0.00*	0.00	**0.00**	2.00	2.30	3.50	0.00					
cow 5	0.00	0.00	*0.00*	**0.00**	3.00	2.60	4.20	0.00						
cow 6	0.00	0.00	0.00	*0.00*	0.82	**2.10**	2.40	3.30	2.10	0.00				
Orsel et al., 2007			***preclinical data determined by first detection in EP fluids***											
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
7358	0.00	0.00	0.00	0.00	***0.00***	0.00	1.48	2.54	2.54	0.00	0.00	0.00	0.00	0.00
7359	0.00	0.00	0.00	0.00	0.00	***1.47***	4.02	2.81	2.24	0.70	0.00	0.00	0.00	0.00
7360	0.00	0.00	0.00	0.00	***0.00***	2.10	2.74	2.90	0.00	0.00	0.00	0.00	0.00	0.00
7361	0.00	0.00	0.00	*0.00*	0.00	0.00	1.35	4.04	4.56	1.44	0.00	0.00	0.00	0.00
7362	0.00	0.00	0.00	0.00	**0.00**	*0.00*	4.80	2.00	0.00	0.00	0.00	0.00	0.00	0.00
7904	0.00	0.00	0.00	0.00	*0.00*	**0.00**	1.96	2.68	2.81	0.00	0.00	0.00	0.00	0.00
7905	0.00	0.00	0.00	0.00	0.00	**0.00**	*4.71*	4.70	2.86	1.97	0.40	0.00	0.00	0.00
7906	0.00	0.00	0.00	0.00	0.00	***0.00***	0.00	5.13	5.40	3.44	0.70			
7907	0.00	0.00	0.00	0.00	0.00	***0.00***	2.74	4.83	2.68	3.13	1.89	0.00	0.00	1.00
7908	0.00	0.00	0.00	0.00	0.00	***0.00***	1.47	4.92	3.44	2.30	1.26	0.00	0.00	1.40
Reid et al., 2006			***preclinical data determined by first detection in nasal swabs***											
UV 59	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Machine Whole	0.00	*0.00*	0.80	0.00	0.00	**0.80**	4.55	3.05	0.55	0.00	0.00		0.00	
UV61	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Machine Whole	0.00	0.00	*0.00*	0.00	0.80	**3.55**	4.05	3.80	3.05	0.55	0.30		0.00	
Burrows et al., 1971			***preclinical data determined by detection in OP fluids***											
			2	3	4	5	6	7	8					
O1 serotype	27		0.00	0.00	*0.00*	**3.30**								
	28		0.00	*0.00*	1.40	3.50	4.60	**4.50**						
	29		0.00	0.00	*1.00*	**5.20**								
	30		0.00	0.00	*1.30*	**2.40**								
A22 serotype	20		0.00	0.00	*0.00*	0.00	**2.80**							
	21		0.00	0.00	***0.00***									
	22		0.00	0.00	*0.00*	0.00	0.00	**1.20**						
	23		0.00	0.00	*0.00*	**1.70**								
Donaldson et al., 1982			***milk titers from preclinical cows***											
preclinical cows		0.55	6.45	3.05	1.35	1.55	0.95							

Legend: FMD studies with in-contact cows, reported virus titers in milk post exposure
EP and OP fluids are esophageal and oesophageal pharyngeal fluids All values in log 10 PFU/ml
Bolded values are on the day cow showed clinical signs
Italicized values indicate the day cows are preclinical
Values listed as 0.00 are non-detections of virus

Analysis for Simulating Milk Titer Values from Preclinically Infections Cows

Overall, 47 data points were available to evaluate the milk titer for preclinically infectious cows with a mean log milk titer being 1.12 $\log_{10}$ PFU/ml. The variability in the FMD titer of milk from preclinically infectious cows was modeled with an exponential distribution based upon Kolomorov Smirnoff goodness of fit test. Given only 47 points, there was a significant uncertainty regarding the overall mean milk titer of FMD from preclinical infectious cows. We used normal (1.12 to 0.25) as the uncertainty distribution of log milk titer from preclinically infectious cows based on central limit theorem (we verified that the sampling distribution of the mean was approximately normal through re-sampling methods). Here 0.25 was the standard error of the mean. The estimate of mean milk titer was then used to parameterize an exponential distribution that represents the variability in milk titer among individual cows. An upper bound Y^{lim} of 5.5 log PFU/ml was utilized to represent the maximum milk titer from the data when one outlier was removed. **Figure D- 1** presents the exponential fit data for the log FMD milk titers in preclinically infectious cows.

We provide the specific steps used in modeling the milk titer in the simulation model below.

For each simulation iteration:

Step 1: Obtain an estimate of mean milk titer of FMD from preclinically infectious cows according to Appendix D equation 1

$$X \sim Normal\ (1.12, 0.25) \qquad \text{Appendix D Eq 1}$$

Step 2: For each preclinically infectious cow j, calculate the milk titer Y_j according to Appendix D equation 2

$$Y \sim Exponential(X), Y^{\lim}\ ) \qquad \text{Appendix D Eq 2}$$

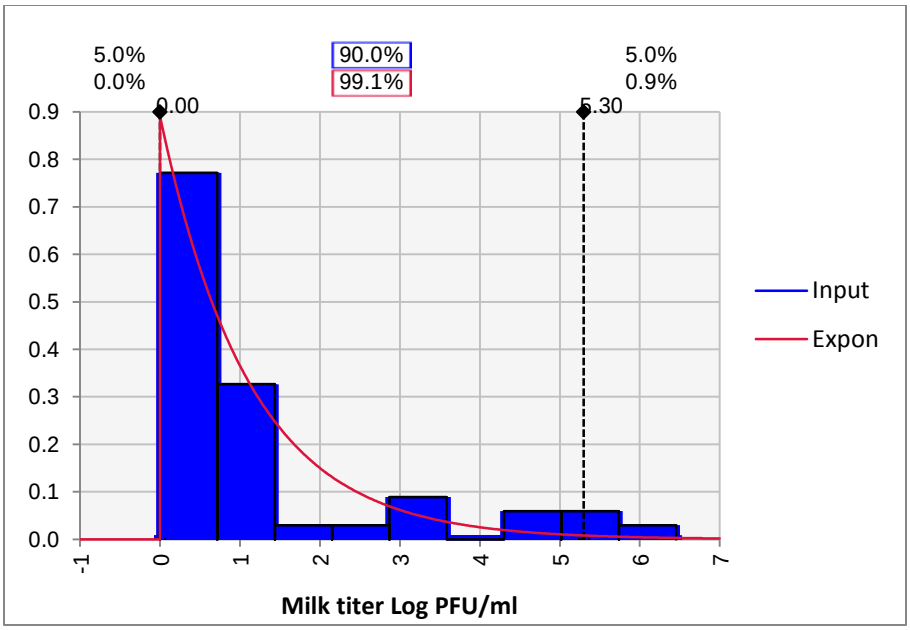

Figure D- 1. Exponential fit for log FMD titer in milk from preclinically infectious cows.

Details for Simulating Milk Titer from Clinically Infectious Cows

Overall, 94 data points were available for the milk titer from clinically infectious cows with a mean of log milk titer being 2.25 log PFU/ml. **Figure D-2** presents the histogram of the raw data. In this case, the milk titer data was not a good fit for standard theoretical distributions when used directly. When milk titer data points with 0 log PFU/ml were removed, the remaining data were a reasonable fit for a Weibull (2.1573, 3.1854) distribution based on Kolmogorov Smirnoff goodness of fit test. An upper bound Z^{lim} of 5.5 log PFU/ml was utilized to represent the maximum milk titer from the data. **Figure D-3** presents the Weibull fit for the log milk titer in clinically infectious cows. We modeled the milk titer in the following three steps:

Step 1: Obtain an estimate of mean fraction *U* of clinically infectious cows that have a milk titer above 0 1og EID_{50}/ml using a beta distribution as shown in appendix D Eq 3

$$U \sim Beta\ (75, 20) \qquad \text{Appendix D Eq 3}$$

Step 2: Obtain an value for the number of clinically infectious cows that have a milk titer above 0 log EID_{50} , as shown it as N^{+} . Here N^{CI} is the number of clinically infectious cows.

$$N \sim Binomial(N^{CI}, U), \qquad \text{Appendix D Eq 4}$$

Step 3: For all N^{+} clinically infectious cow with a viral titer above 0 log PFU/ml, calculate the milk titer Z_k according to Appendix D equation 5

$$Z_k \sim Min(Weibull(2.157, 3.185), Z^{lim}) \qquad \text{Appendix D Eq 5}$$

The approach utilized to simulate FMD titer in milk from clinically infectious cows is similar to the use of zero inflated probability distributions.

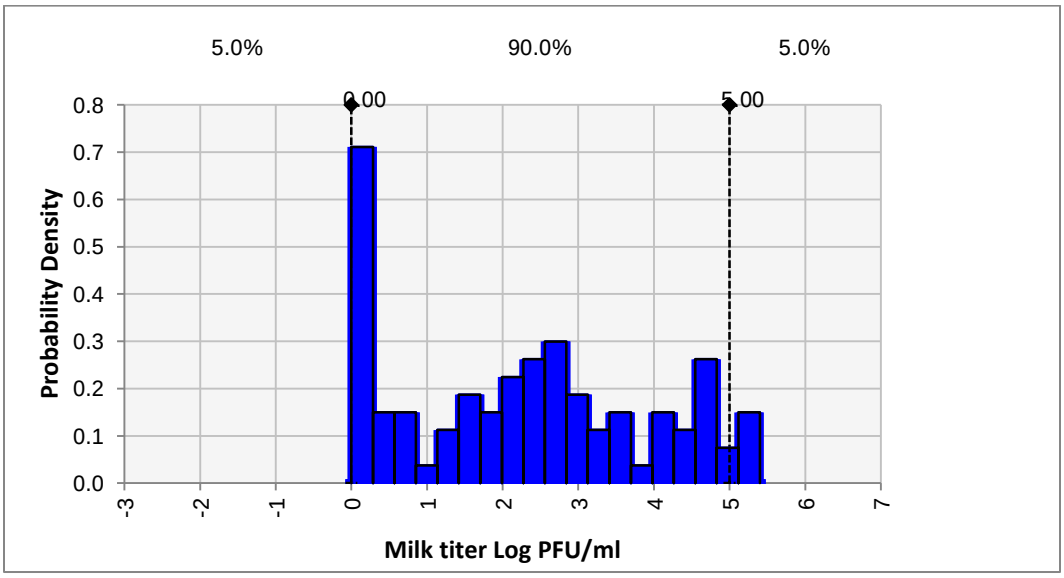

Figure D-2. Histogram log FMD titer in milk from clinically infectious cows based on raw data.

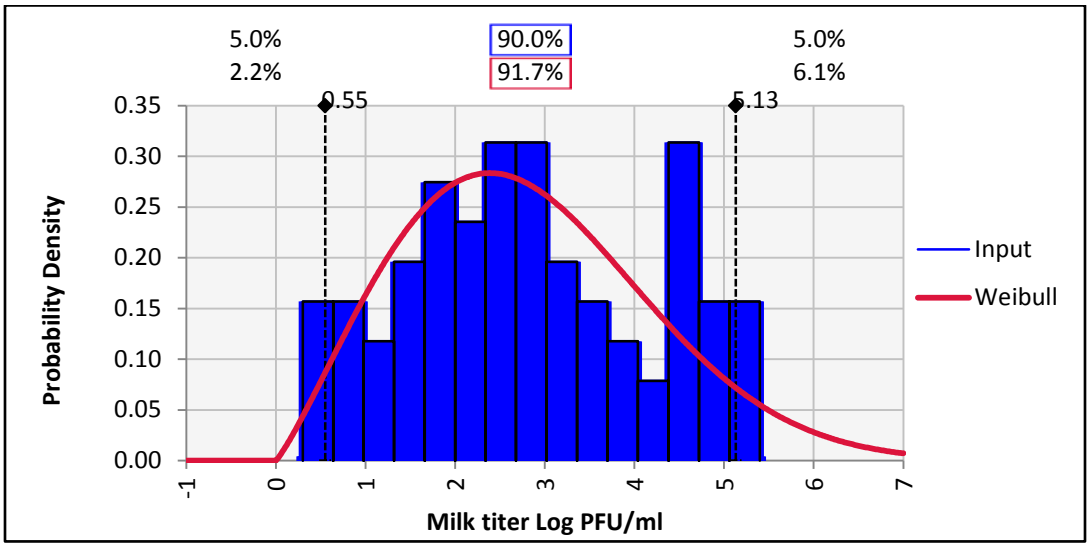

Figure D-3. Weibull fit for log FMD titer in milk from clinically infectious cows to data set in which data points with 0 log PFU/ml have been removed.

Appendix E. Bioaerosol Science

Sources and Generation

Generation of bioaerosols can occur under natural conditions as well as from human activities such as spreading of slurries, pressurized spray irrigation, and aeration basins at wastewater treatment plants. In general, airborne microorganisms (bacteria, fungi, and viruses), and their components, are generated as a mixture of droplets or particles, having different aerodynamic diameters ranging from 0.5 to 100 µm (Cox and Wathes, 1995; Lighthart, 1994). Microorganisms associated with droplets evaporate to dryness or near-dryness before impacting the ground or vegetation and are transported by air currents (Dungan, 2010). The optimum aerodynamic particle range which represents a hazard to the human respiratory tract is between 1.0 and 10 µm (Mohr, 2005, 2007).

The dissemination and transport of bioaerosols depends on the method of bioaerosol generation and energy input into the system. Pressurized air, electricity, centrifugal forces, impaction, or heat can provide the energy needed to produce small particles. Many of these forces are so violent that inactivation of the microorganisms will occur. Fluids associated with newly aerosolized particles will instantaneously start to evaporate. The distribution and concentration of particle sizes are two important variables that directly affect the potential for dissemination and transport.

Transport

The transport, behavior, and deposition of bioaerosols are affected by their physical properties (i.e., size, shape, and density) and meteorological factors they encounter while airborne. Naturally occurring bioaerosols are ejected into the atmosphere by wind, rain and bursting bubbles, and other processes. The environmental conditions of wind velocity, RH, temperature, and precipitation significantly affect transport of bioaerosols with atmospheric stability being a major factor (Jones and Harrison, 2004; Lighthart, 2000).

Bioaerosols are subject to inactivation and transport the moment they become airborne. Naturally occurring bioaerosols are ejected into the atmosphere by wind, rain and bursting bubbles, and other processes. Particle sizes of droplets are usually small (2 to 10 µm) and they tend to follow the streamlines of the local wind. Particles with sizes smaller than 5.0 µm act as vapors and follow the streamlines of the airstream. The aerodynamic diameter of particles determines whether it is small enough to follow the streamlines of the surrounding flows, or if it is large enough to cross streamline flow and impact upon a surface. Deposition of larger aerosols occurs through gravitational settling, impaction, diffusion, convection (due to temperature variations), and wash-out by raindrops (Muilenberg, 1995).

Viability, Stability, and Infectivity

The viability of bioaerosols is dependent upon their chemical makeup and the environmental and meteorological factors they are exposed to, such as wind speed, temperature, and RH. These atmospheric conditions are strongly influenced by features such as large-scale flow fields, geographical locations, and local topography. The most significant environmental factors influencing viability are RH, solar irradiance, temperature, and oxygen concentration. Additional influences include air ions and open-air factors (OAF). Atmospheric turbulence is responsible for diffusion of particles during transport by the wind (mean wind speed) and is strongly influenced by local atmospheric conditions and the diurnal variation of solar irradiance reaching the ground.

Of all of the measurable meteorological parameters, RH is the most important with respect to aerosol stability, which is an important determinant of bioaerosol viability and infectiousness (Mohr, 2005, 2007, 2011). The majority of airborne microorganisms are immediately inactivated upon release because of environmental stresses (desiccation, temperature, and oxygen) which act upon and alter the surface of the microorganism. The fundamental factors that affect the viability of microorganisms are the state of the water and water content of the bioaerosol. As RH decreases, the water available to the exterior environment of the microorganism also decreases. Loss of water can cause dehydration, resulting in inactivation of many microorganisms. The RH of the system also directly affects the density of the bioaerosol unit. The size, shape, and density of the aerosolized particles are directly related to the aerodynamic diameter, which determines settling velocity and location of deposition in the respiratory tract (Mohr, 2005, 2007).

Studies to determine the effect of temperature on the fate of bioaerosols have generally shown that increasing temperatures tend to decrease the viability of airborne microorganisms (Dimmock, 1967). It is difficult to separate the effects of temperature and RH, as the vapor pressure and RH of a system are dependent on temperature. The lipid content of the outer coat, or capsid of a virus, determines the stability at high or low RH values. Viruses in bioaerosols are generally inactivated by water loss and conformational changes in the protein coat. In research studies with artificially generated plague bioaerosols, the bacteria were stabilized by adding sugars to the solution (Mohr, 2011). Skim milk has been shown to be a stabilizer; therefore, milk – or its components – may be protective of the virus (personal communication with J.A. Mohr, 2011).

Bioaerosol Experts Interviewed and Questions

The seven experts who responded to a request for expert opinion were interviewed about the probability of generating milk bioaerosols during pumping and transport of raw milk. Background information was provided to the experts on tanker design, air vent design, milk chemistry, milk spillage, and FMDv milk studies. The following questions were posed to the experts. **Table E-1** summarizes their responses.

1. Will pumping of milk into the tanker produce milk aerosols that can escape the tanker? Will they be small enough to remain in the airstream and escape the tanker without impacting the inside of the tanker, the air vent (Runovent) or the dome lid?

2. While milk is pumped into the tanker, the air within the tanker moves through a centrifugal/baffle shaped air vent and may hit the top of the circular dome lid cover. Would a significant portion of the aerosols in the tanker air be deposited either by hitting the vent baffles or the dome lid while air is released to the outside?

3. Would you expect significant aerosolization of milk within a partially filled tanker during transportation, due to movement of milk?

4. If yes, would you expect a significant portion of the aerosolized particles to be less than 10 µm in size?

5. If you had a mist of aerosols above the milk level in a tanker, could they move into the airstream and out of the tanker during the pumping at a subsequent farm?

The following experts that agreed to an interview have varying backgrounds in aerosol /bioaerosols research including microbiology, engineering, aerosol physics, and bioterrorism agents. The responses from the Drs. Brian Bennett and Russell Bartholomew at Dugway Proving Ground are not included in this table, as their answers did not fit the table format.

- **Mark Buttner**, PhD, University of Nevada, Las Vegas Department of Environmental and Occupational Health
- **Thomas Kuehn**, PhD , University of Minnesota, Department of Mechanical Engineering
- **Jeff Allan Mohr**, PhD, Special Programs Division, US Army Dugway Proving Ground, Dugway, UT
- **Peter Raynor**, PhD, University of Minnesota, Division of Environmental Health Sciences
- **John Volckens**, PhD, Colorado State University, Department of Environmental Health and Radiological Sciences
- **Brian Bennett**, PhD and **Russell Bartholomew**, PhD, Dugway Proving Ground US Army Aerosol Branch Life Sciences Dugway, UT

Table E-1. Summary of bioaerosol responses from experts except Dugway Proving Ground group.

Questions posed to the experts	Can bioaerosols be generated during pumping that can escape the tanker at the first farm?	Will aerosols move through the runovent and exit the tanker?	Will there be aerosolization of milk within the tanker during transport?	Milk leakage out of the tanker - will aerosols be generated?	Will bioaerosols be generated while pumping milk at the second farm?
Expert 1	**Low Probability** due to milk temp and pumping into bottom of tanker		**Possible** Transport will produce more aerosols than pumping. The majority of the mass will be large particles.	**Possible** Road sheer and wind shear could cause re-aerosolization	
Expert 2	**Unlikely** to generate particles in the threat range (1-5um)	**Unlikely**	**Yes** Could form a mist within the head space	**No** Wind sheer will not lead to bioaerosols **Some** Road Sheer bioaerosols production	**Possible** If aerosolized mist is in headspace, may not settle down quickly prior to pumping
Expert 3	**Unlikely** Most aerosols will be large in size and settle out	**Not Likely** Vent is impaction device	**No** Not enough energy available	**No** Unless it is ejected under pressure **Possible** Road sheer	**No Concern** Unless aerosols are in the headspace
Expert 4	**Unlikely** But small aerosols could be produced	**Possible** Depends on where the air is exiting through the Runovent® or under the dome lid. It is unlikely that all particles will be collected by impaction.	**Unlikely** Mostly large droplets will be formed and fall back into liquid	**Low Probability**	**Low-negligible**
Expert 5	**Possible** Aerosols will be produced, but the size and distribution of the population is unknown	**Possible** Aerosols can be produced, questionable if they can escape and population size	**Possible** If air can freely leave the tanker during transport aerosols could be produced	**Possible, but unlikely** Maybe 1% of particles can escape **Yes** Resuspension from the road	**Not probable** Less concerned as pumping is under existing fluid level