Risk Assessment: Transmission of foot and mouth disease virus in milk droplet aerosol generated during milk tanker collection.

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1. Executive summary

This risk assessment examines the potential transmission of foot and mouth disease virus (FMDV) through the generation of milk droplet bioaerosol during the collection and filling of milk tankers on farm. FMDV virus is known to spread between animals by the aerosol route and has been shown to be able to spread some distance with favourable climatic conditions.

The ability of FMDV to transmit between animals by the aerosol route is affected by multiple factors including weather (air temperature, wind, humidity, precipitation), strain of FMDV, animal species, animal susceptibility, animal density, distance from source of plume and particle size of aerosol droplets.

Factors that affect the ability of milk to form infectious bioaerosol droplets include viscosity of milk, protein and fat content of milk, titre of FMDV in milk (dilution effect), temperature and humidity (in tanker head space) of milk in the milk tanker and the pressure relief valve or air vent construction.

From the available data it is assessed that the likelihood of milk being aerosolised during the filling of a milk tanker, forming a sufficiently dense plume of droplets that can escape the milk tanker vent, and the plume travelling far enough to settle and constituting a high enough dose to be infective for a group of animals is negligible.

2. Introduction

This risk assessment examines the potential transmission of FMDV through the generation of milk droplet bioaerosol during the pumping, collection and transport of milk from the on farm bulk milk tank to the milk tanker.

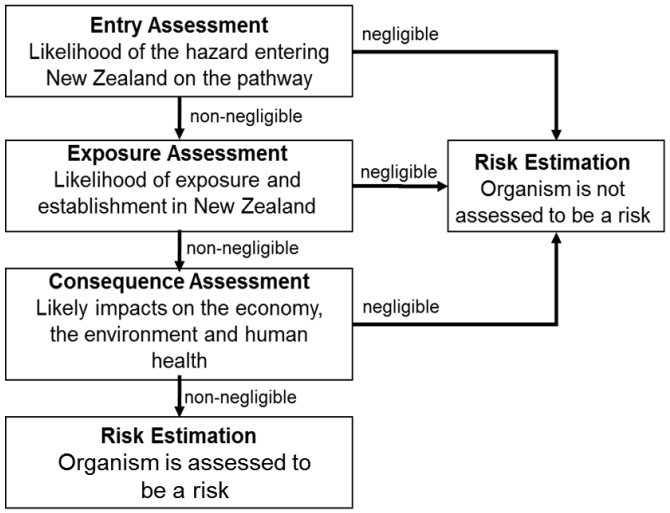
This assessment examines the likelihood that milk is collected from an infected farm and when milk is subsequently collected at an unaffected farm, the pumping of milk and filling of the milk tanker creates a milk droplet bioaerosol that could disperse through the tank vent or pressure relief valve.

The risk assessment is conducted as part of the New Zealand emergency disease contingency planning for foot and mouth disease (FMD) and is to assist in the planning and preparation of protocols to be applied should an outbreak of FMD occur in New Zealand.

3. Methodology

The methodology used in this risk assessment is adapted from the import risk analysis guidelines as described in Biosecurity New Zealand Risk Analysis Procedures – Version 1 (Biosecurity New Zealand 2006). This methodology takes into account, and is based on the recommendations made in Section 2 of the *Code*, summarised in Figure 1.

Figure 1: Import risk assessment process



Import risk assessment consists of:

Entry assessment: The likelihood of a hazard (pathogenic organism) being imported with the commodity.

Exposure assessment: Describes the biological pathway(s) necessary for exposure of susceptible animals or humans in New Zealand to the hazard. Further, a qualitative estimation of the probability of the exposure occurring is made.

Consequence assessment: Describes the likely consequences of entry, exposure and establishment or spread of an imported hazard.

Risk estimation: An estimation of the risk posed by the hazard. This is based on the entry, exposure and consequence assessments. If the risk estimate is assessed to be non-negligible, then the hazard is assessed to be a risk and risk management measures may be justified to effectively manage the risk.

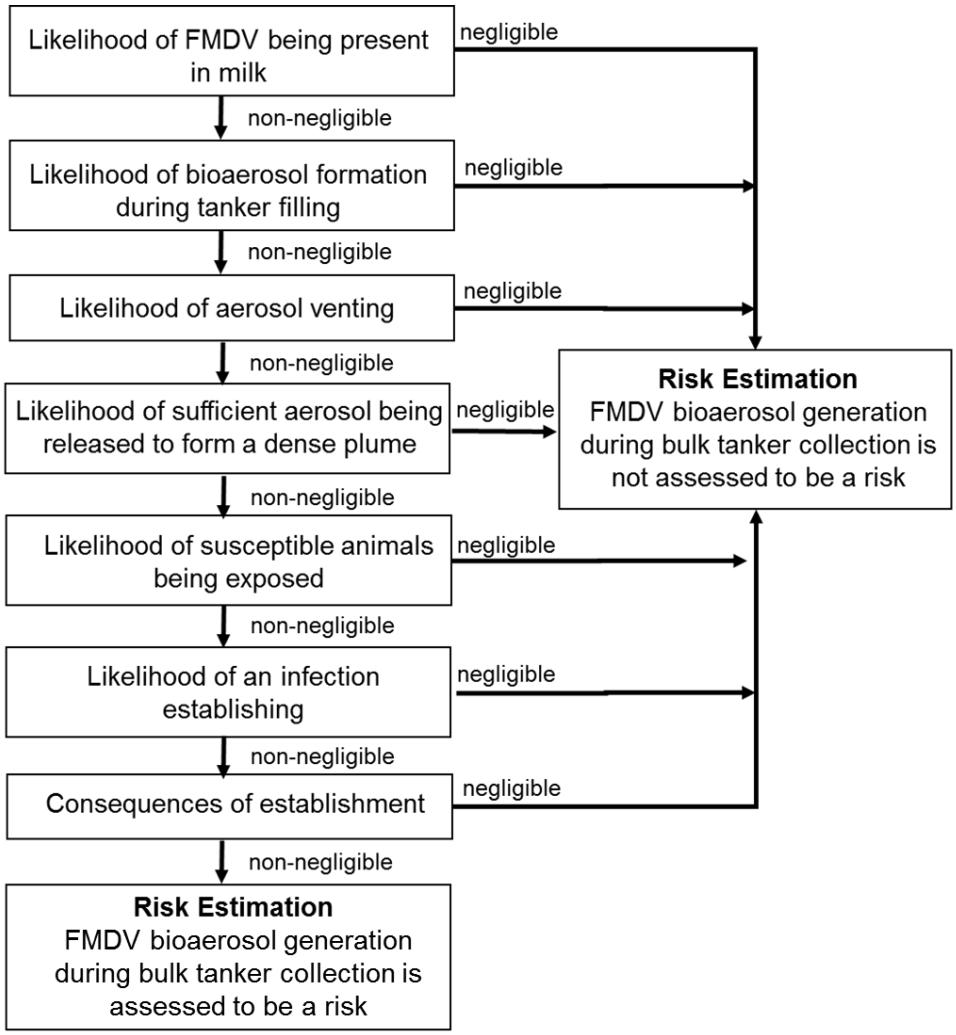
Not all of the above steps may be necessary in all risk assessments. The OIE methodology makes it clear that if the likelihood of entry is negligible[1] for a certain hazard, then the risk estimate is automatically negligible and the remaining steps of the risk assessment need not be carried out. The same situation arises when the likelihood of entry is non-negligible but the exposure assessment

[1] Negligible and non-negligible are terms used as adjectives to qualify risk estimates. Negligible is defined as not worth considering; insignificant. Non-negligible is defined as worth considering; significant. Very low as a risk description means close to insignificant. Low means less than average, coming below the normal level. Medium means around the normal or average level, and high means extending above the normal or average level (Biosecurity New Zealand 2006).

concludes that the likelihood of susceptible species being exposed is negligible, or when both entry and exposure are non-negligible but the consequences of introduction are assessed to be negligible.

For the purposes of this risk assessment, the risk assessment process has been adapted as summarised in Figure 2.

Figure 2: Risk assessment process to assess the risk of FMDV milk droplet aerosol generation during bulk tanker collection



4. Foot and mouth disease virus

4.1. HAZARD IDENTIFICATION

4.1.1. Aetiological agent

Family: *Picornaviridae*. Genus: *Apthovirus*.

There are seven serotypes of the virus: O, A, C, SAT1, SAT2, SAT3 and Asia 1 (OIE 2015a).

4.1.2. OIE list

FMD is listed under diseases affecting multiple species (OIE 2015c).

4.1.3. New Zealand status

FMD is an exotic notifiable disease that has never occurred in New Zealand.

4.1.4. Epidemiology

FMD is a highly contagious viral disease with a significant economic impact, affecting all cloven hoofed animals, both domestic and wild (Thompson *et al.* 2002). Cattle are the main host but sheep, goats, swine and buffaloes are susceptible (CFSPH 2015). Among the *Camelidae*, only Bactrian camels (*Camelus bactrianus*) are susceptible (OIE 2015b). Dromedary camels (*Camelus dromedariues*) do not appear to be susceptible (OIE 2015b, CFSPH 2015). New world camelids, llamas and alpacas, have only been shown to be susceptible by experimental infection and are not thought to play an epidemiological role in the disease (OIE 2015b, CFSPH 2015). Apart from African buffaloes (*Syncerus caffer*), wildlife species have not been shown to act as a reservoir (OIE 2015a, b).

Different strains of FMDV may have little to no infectivity to bovids e.g. pig adapted strain of type O lineage (designated O/Taw/97) is infective to pigs but cattle do not develop clinical signs or viraemia. There may also be a significant variation between the different strains in the amount of virus excreted by infected animals (Thomson and Bastos 2004). Therefore predicting the titre of virus present in milk is difficult.

FMD is widespread, occurring endemically in areas of South America (Correa *et al.* 2002), Africa and Asia (OIE 2015a).

FMD has an incubation period ranging from 2-14 days and the *Code* defines it as 14 days when making recommendations to manage the disease (OIE 2015b).

Clinical signs vary with the strain of FMDV, exposure dose, age and breed of animal, host species and degree of host immunity (Thomson and Bastos 2004, OIE 2015a). Clinical signs may vary from inapparent to severe. Morbidity rates may be as high as 100% but mortality is generally low in adult animals (1-5%) and may be higher in calves, lambs and piglets (>20%) (OIE 2015a). Recovery is often uncomplicated and usually takes about 2 weeks (Davies 2002, Thomson and Bastos 2004, OIE 2015a). Sheep and goats may show subclinical disease whilst infectious (Alexandersen *et al.* 2003) and pigs are an important amplifying host (OIE 2015a).

Transmission of FMDV may be direct or indirect. Direct transmission, associated with contact between infected animals and susceptible animals or contaminated animal products, is the most common pathway of spread (Donaldson 1987, Alexandersen *et al.* 2003). Indirect transmission is associated with contact with contaminated objects (hands, foot wear, milking machines), consumption of contaminated meat and animal products (milk), artificial insemination, airborne spread and humans (who may harbour the virus in the respiratory tract for 24 to 48 hours) (Gloster *et al.* 1982, Sutmoller *et al.* 2003).

Sources of FMDV include incubating and clinically affected animals (Sanson 1994, OIE 2013). Carrier animals are defined as recovered, vaccinated or exposed animals in which FMDV persists in the oropharynx for more than 28 days (Sutmoller *et al.* 2003, OIE 2013). Carrier rates in cattle vary from 15-50% and carrier status usually does not persist for more than 6 months although some cases may extend to 3 years (OIE 2013). African buffaloes may harbour virus for up to 5 years and are the main reservoir for SAT serotypes (Thomson and Bastos 2004, OIE 2013).

The significance of carrier animals in the transmission of FMDV is still unclear, the only available evidence suggesting transmission of disease between African buffaloes and cattle (Thomson and Bastos 2004).

Airborne spread of FMDV

Airborne spread of FMDV is associated with respiratory aerosol droplet and droplet nuclei generated from nasopharyngeal secretions of infected animals (Alexandersen *et al.* 2003). Airborne FMDV has been associated with particles of various sizes with the greatest infectivity (65-71%) associated with particles in the range of 6 µm and 19-24% with particles 3-6µm (Donaldson 1986). Aerosol particles produced by pigs ranged from 0.015 to 20.0µm (Gloster *et al.* 2007).

The most efficient producers of aerosol virus are pigs and the most susceptible to airborne infection are cattle (Thomson and Bastos 2004). Simulations suggest that 100 infected pigs could infect susceptible cattle 6 to 90 km downwind and 1000 pigs could create an aerosol plume capable of infecting cattle over a distance of 300 km (Sørensen *et al*. 2000, Donaldson and Alexandersen 2002). Modelling of disease transmission from infected cattle or sheep suggest that infection is unlikely to occur over distances greater than 3 km (Sørensen *et al*. 2000).

In the review by Alexandersen *et al.*, (2003), the impact of particle size on distance of spread and localisation in the respiratory tract is discussed. Larger droplets (>40µm) are likely to settle out quickly in still air, although turbulence would keep them suspended for longer. Larger particles (>10µm) are likely to settle in the upper respiratory tract (nares or sinuses) on inhalation, with medium sized particles (4-10µm) extending to the nasopharynx, trachea and bronchi and small particles extending to bronchioles and alveoli (optimal 1-4µm) (Alexandersen *et al.* 2003, Herman 2012). The primary site of FMDV replication is the nasopharynx (Stenfeldt *et al.* 2015) therefore particles settling in this area have a higher possibility of initiating infection.

The likelihood of disease transmission through windborne aerosol is dependent on the strain of FMDV, species of excreting animal, number of infected animals, distance to nearest susceptible population, number of animals in a susceptible population and species of susceptible animal (Sellers and Forman 1973, Sørensen *et al*. 2000, Donaldson and Alexandersen 2002).

Other factors that favour windborne dissemination of airborne virus are flat terrain, high humidity, low precipitation and low to moderate wind speed (Sørensen *et al*. 2000, 2001). The optimal relative humidity (RH) of air for the survival of FMDV in respiratory aerosols is >55% (Donaldson 1986).

Physiological fluids such as milk, faeces and slurry have a protective effect on the decay of FMDV (Donaldson 1986). The spread of FMDV through bioaerosols from milk, urine, faeces and slurry has been suggested (Donaldson 1997, Alexandersen *et al.* 2003).

FMDV in milk

FMDV is excreted in milk (Burrows 1968, Dawson 1970). FMDV is released into milk through exocytosis by a membrane-limited vesicle, sloughing of infected epithelial cells, merocrinal secretion and association of virus with casein micelles, and either inclusion of the virus within the fat globule or attachment to the plasma membrane that surrounds the fat globule (Blackwell *et al.* 1981, Blackwell *et al.* 1983a and b).

During a FMDV outbreak infected animals may be producing infected milk for 1-4 days before showing clinical signs (Burrows 1968, Sanson 1994). This is the period of highest risk for disease transmission between infected and un-infected premises. The mechanisms through which milk is suggested to cause infection include ingestion of milk, inhalation of milk droplet aerosol and contamination of humans and equipment.

The majority of references in the literature implicating milk in outbreaks of FMDV is associated with the movement of milk tankers and people rather than the direct feeding of milk or milk products. There are two instances that are widely referred to: Brooksby (1959) refers to the feeding of 'infected' milk to calves in transit at a collection point in Crewe, England, before the calves where moved to various locations and subsequently are implicated either directly or indirectly in 101 new outbreaks. Henderson (1968) references an outbreak of FMDV in three piggeries that were fed using semi-skimmed milk supplied from one milk tanker. It is not stated if this milk was heat treated prior to feeding or whether the milk tanker may have been contaminated.

Titres of FMDV in milk

There are few published studies on the titres of FMDV shed in milk during an outbreak (Spickler and Roth 2012). The main reference is the 1967-68 outbreak in the United Kingdom and titres of virus was reported to vary from trace to $10^{6.6}$ tissue culture infectious dose$_{50}$/ml ($TCID_{50}$/ml) in milk samples of individual animals (Hedger and Dawson 1970).

During the 1967-68 United Kingdom outbreak, three samples were taken from different bulk milk tanks. One bulk tanker contained $10^{3.75}$ $TCID_{50}$/ml of FMDV in 2,070.6 litres, including 829 litres from a farm where one cow out of 107 was infected. A second bulk tanker with $10^{4.0}$ mouse ID_{50}/ml contained 4,618.2 litres and had collected milk from 8 infected cows out of 75 producing 514.8 litres. A sample from a farm storage tank contained milk from three infected individuals from a herd of 55 cows and had a viral titre of $10^{4.5}$ $TCID_{50}$/ml (Hedger and Dawson 1970).

4.1.5. Hazard identification conclusion

FMDV is excreted in milk before the appearance of clinical signs and can be present at high titres in the milk of individual animals, sufficient to cause infection in susceptible animals via the oral route.

FMDV in milk bioaerosols is identified as a potential pathway for disease transmission.

4.2. RISK ASSESSMENT

4.2.1. Likelihood of FMDV being present in milk

FMDV is excreted in the milk of infected animals and during an outbreak infected animals may be producing infected milk for 1-4 days before showing clinical signs (Burrows 1968, Sanson 1994). This is the period of highest risk for disease transmission between infected and un-infected premises. FMDV will be present in the milk of infected herds before evidence of clinical signs and their milk will contribute to the total volume of milk produced on farm, therefore the likelihood of FMDV being present in milk is non-negligible.

4.2.2. Likelihood of milk bioaerosol formation occurring during milk collection

Particles in the range of 1 - 10µm are considered to be the main threat with regards to the ability to remain airborne, be inhaled and transmit disease (USDA 2015).

Information in the literature and the consensus of opinion by a panel of experts, consulted by the USDA to derive a baseline risk analysis regarding the risk of FMDV in the movement of raw milk, agreed that at least 90% of the mass of milk bioaerosols generated during the collection and movement of milk will be composed of larger droplets in the range of 100µm (Herman 2012, USDA 2015). This is based on several factors including:

- A significant amount of energy is required to produce droplets less than 10µm (USDA 2015). Apart from respiratory aerosols, aerosols in the range of 1 to 10µm are mostly produced through the application of various mechanical means i.e. high pressures expelling fluid through fine nozzles, 'spinning tops', nebulizers, boiling etc.
- Chilling of bulk tank milk (at least 7°C or below, MPI 2015) which is likely to increase the viscosity of the milk and decrease the likelihood of aerosol generation (USDA 2015).
- The percentage of milk solids in the milk; this affects the viscosity and density of the milk. Higher milk solid percentages decreases the ability for aerosol formation through the formation of larger or denser droplets that settle out rapidly (USDA 2015).
- At low temperature, (<10°C), the viscosity of milk, protein and fat content of milk will increase droplet size and decrease the rate of evaporation of the droplets, promoting rapid settling of droplets and decreased ability to form droplet nuclei (evaporated droplets retaining infectious organisms in a particle nature, dust 0.1-10µm, that can remain airborne for extended periods of time) (Herman 2012, USDA 2015). Larger droplets will fall back rapidly into milk or condense on the sides of the tank.
- Relative humidity and temperature in the head space (assumed to be similar to that of the chilled milk) within the milk tanker (USDA 2015). High humidity levels (greater than 50%) and low temperatures result in the formation of larger and fewer droplets with droplets falling back rapidly at even temperatures (USDA 2015). This also allows for rapid settling of droplets once the vehicle is stationary, prior to further filling of the tank (USDA 2015).
- Filling of tankers from the bottom creating less turbulence, bubble formation and splash in the tank (USDA 2015). Expert opinions elicited by the USDA 2015, agreed that once filling of milk occurs below a fluid layer the risk of aerosol generation is negligible.
- 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 (USDA 2015).
- Ninety degree bends in vents will eliminate a proportion particles in the range of 1 - 10µm as a result of impaction (USDA 2015).

The majority of New Zealand milk carriers are bottom filled although trailers are top filled (personal inspection[2]) and milk vents are closed during tanker movement and open only when filling the milk tank.

The ability of chilled milk to form a fine bioaerosol, between 1 – 10µm, is assessed to be very low, although there may be some fine droplets within the headspace of the tank which could be expelled during filling. The likelihood of milk bioaerosol formation is assessed to be very low.

4.2.3. Likelihood of milk bioaerosol venting from the tank

The New Zealand milk tanker pressure relief valve system has the ability to contain milk in tankers following accidents and 'rollover' (personal inspection[2]). The seal integrity is extremely high and there is no risk of aerosol egress during transit. Release of milk bioaerosol during milk tanker movement is not a risk due to efficiency of vent closure.

The first or second filling of the milk tanker trailer (which is top filled) has the highest risk of generating bioaerosols, filling of an empty main milk tank or milk tank with very low filling is considered the second area of risk. Filling of an empty tank will not be a risk for the farm as it is the only milk in the tank but there could be a very small fraction of fine bioaerosols that remain airbourne in the tanker head space which could be vented at the second collection.

New Zealand milk tanker vehicle inspection ports are located on the sides and back of the tanks, therefore opening of the inspection ports during pumping of milk is not possible. All venting and aerosolization that could occur would be through a single milk vent, located on top of the tank.

Once stationary there is at least 2 minutes or more during which milk settles in the tank and air is cycled through the vent mechanism before the vent is opened for pumping (personal inspection[2]). During this period the effect of gravity, collision and coagulation of droplets and particles of different sizes and condensing on the sides of the tanks should result in the settling out of aerosols, although a small fraction of fine aerosols may persist (USDA 2015).

The likelihood of milk bioaerosol venting from the milk tanker is assessed to be very low.

4.2.4. Likelihood of bioaerosol forming a plume outside the tanker

The vast majority of droplets produced (assumed 90%) will vary in a range from at least 50 to 200µm and these are unlikely to escape the milk tanker vent.

The ability of chilled milk to form a fine bioaerosol, between 1 – 10µm, is assessed to be very low, although there may be some fine droplets within the headspace of the tank which could be expelled during filling. However a panel of aerosol experts suggest that a bend of 90° or more in the milk tanker vent will remove a large component of particles in the range of 1 -10µm through impaction (USDA 2015). The potential number of fine bioaerosols that could be released is unknown, however experimental seeding of milk with *Bacillus globigii* spores (Harper, unpublished, 1968 cited by Donaldson 1997 and USDA 2015), and measuring the number of spores expelled through milk tanker

[2] Personal visit to Fonterra Te Rapa with visual inspection of milk vents and tankers, accompanied milk tanker collection and discussions with technical staff.

vents during filling suggests that the 'amount of FMDV likely to be aerosolised and dispersed by this manner is negligible' (Donaldson 1986, 1997).

Where fine milk bioaerosol droplets are released, the bioaerosol will be affected by multiple factors. Fine aerosol is likely to be expelled under some pressure associated with the rapid filling of the tank which will result in rapid dispersion and diffusion of fine droplets as they exit the vent. Furthermore the released fine aerosols will be subjected to many external factors that will affect the spread and density of the aerosols including; air temperature (high air temperatures may result in rapid desiccation), rain, wind (wind speed, direction) air turbulence (in conditions or environments favouring turbulence there will be rapid dispersion of droplets and break up of plumes or aerosol clouds), topography of surroundings (buildings, trees, hills, vehicles; all factors that increase turbulence in air surrounding the tanker, and likely contact with animals) and RH (very high RH will result in in droplets taking on water, increasing the size and density of droplets resulting in rapid settling. At very low RH's there can be very rapid desiccation of droplets which could inactivate virus).

The likelihood that milk will form a fine bioaerosol and be expelled from the milk tanker with sufficient density to form a plume is assessed to be negligible.

4.2.5. Risk estimation

The likelihood that milk will form a fine bioaerosol and be expelled from the milk tanker with sufficient density to form a plume is assessed to be negligible. Pumping of milk on farm is not assessed to be a risk for FMDV transmission.

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