



Rapid Risk Assessment Miscellaneous egg products for human consumption

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Rapid risk assessment:

Miscellaneous egg products for human consumption



Technical advice document providing risk assessment recommendations for “100 year old duck eggs”, drinks containing eggs, muscle protein powders containing eggs, and non shelf-stable products containing eggs.

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Approved for general release

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Contents

Page

1.	Executive summary	1
2.	“100 year old” duck and chicken eggs	2
3.	Drinks containing eggs	6
4.	Muscle protein powder containing egg from all countries	19
5.	Non shelf-stable food containing up to 100% egg and frozen poached eggs	25
6.	References	33

1. Executive summary

Currently, a number of egg products are imported into New Zealand for human consumption. The measures permitting importation are not based on a risk analysis so it has been requested that the biosecurity risks be assessed for selected egg products.

All exotic pathogens associated with chicken and duck eggs were identified from previously published Ministry for Primary Industries risk analyses. A pathogen is defined as exotic if it is not found in New Zealand, or if a more virulent strain exists overseas.

For “100 year old” duck and chicken eggs all exotic pathogens were assessed to be inactivated by the manufacturing process which involves a prolonged period of exposure to salt and a high pH environment. No biosecurity risks have been identified for this commodity.

Exotic avian influenza viruses, Newcastle disease virus, group 1 adenoviruses and exotic *Salmonella* spp. were identified as hazards in imported drinks containing egg (eggnog and advocaat). The risk assessments concluded that the risk was negligible for all these pathogens due to inactivation via the manufacturing process, or because of the negligible likelihood of susceptible avian species being exposed to these drinks.

For muscle protein powders containing eggs, only exotic avian influenza viruses and group 1 adenoviruses were identified as hazards. A risk assessment for each pathogen concluded that there is a negligible likelihood of exposure to susceptible avian species and no biosecurity risks have been identified for this commodity.

Finally, for non shelf-stable food containing up to 100% egg and frozen poached eggs, group 1 adenoviruses and *Mycoplasma iowae* were identified as hazards. *M. iowae* was not assessed to be a risk due to a negligible likelihood of entry. Group 1 adenoviruses were assessed to be a risk in both commodities and risk management options are presented.

2. “100 year old” duck and chicken eggs

2.1 INTRODUCTION

Alkalised duck eggs are routinely imported into New Zealand as a shelf stable product. Previously, a rapid risk analysis (MPI 2009b) assessed this commodity and concluded that no risk management measures were required. As part of ongoing import health standard development, the Animal Imports team has requested a further assessment be completed.

2.2 COMMODITY DEFINITION

The commodity is defined as duck or chicken eggs that have been subject to an alkalisation process. Traditional methods of alkalisation involve coating the egg in a paste (usually containing salt, calcium hydroxide and sodium carbonate) and commercial methods usually soak eggs in a brine solution containing 4.2% sodium hydroxide (NaOH), 5% salt and 2% Chinese tea and a divalent cation, which was traditionally lead but due to food safety concerns zinc (0.2%) is now more commonly used.

The diffusion of NaOH through the semi-permeable eggshell causes the internal contents of the egg to harden, with the pH of the albumen increasing to greater than 11 and egg yolk to greater than 10 (Ganasen and Benjakul 2010; Ganasen and Benjakul 2011). The curing time can be variable with a minimum duration of 3 weeks (Benjakul and Ganesan 2015).

2.3 PRELIMINARY HAZARD LIST

2.3.1 DISEASES/AGENTS EXOTIC TO NEW ZEALAND THAT ARE LIKELY TO BE ASSOCIATED WITH EGGS

Previously published import risk analyses for chicken egg powder and hatching eggs of domestic ducks and chickens (MPI 2008a; MPI 2009a; MPI 2012) identified pathogens that are exotic to New Zealand and likely to be associated with eggs.

Based on the findings of these previous assessments, the following list of pathogens is the preliminary hazard list for this commodity:

- Avian influenza viruses (Orthomyxoviridae)
- Avian leukosis/sarcoma (Retroviridae)¹
- Avian paramyxovirus type 1 (Newcastle disease), 2 and 3²
- Campylobacteriosis (*Campylobacter jejuni* and others)³
- *Chlamydophila psittaci*⁴
- Duck enteritis virus (anatid herpesvirus 1)
- *Escherichia coli*⁵
- Goose and Muscovy duck parvoviruses (Derzsy's disease)
- Group 1 adenovirus infection (Angara disease)

^{1,3,4,5} Although exotic strains of this organism was previously identified as a hazard (MPI 2008), a subsequent assessment has concluded that these exotic strains are no more virulent than those present in New Zealand (MPI 2009a), so no further assessment is required here.

²Avian paramyxovirus type 2 and 3 are no longer considered pathogenic and transmission in eggs has not been demonstrated (Australian Department of Agriculture, Fisheries and Forestry 2013).

- Infectious bronchitis (Coronaviridae)⁶
- Muscovy duck reovirus
- Mycoplasmosis (*Mycoplasma iowae*)
- *Ornithobacterium rhinotracheale*
- *Salmonella* Arizonae
- *Salmonella* Enteritidis
- *Salmonella* Gallinarum-Pullorum
- *Salmonella* Typhimurium DT104

2.3.2 DISEASES/AGENTS INACTIVATED BY PROCESSING CONDITIONS

Avian influenza

The Food and Agricultural Organisation (no date) recommends that a 2% solution of NaOH may be used to inactivate avian influenza virus in organic matter, such as poultry manure when applied for 10 minutes.

Avian influenza viruses have been shown capable of surviving when being subjected to a pH of 12 for up to 24 hours (strain H7N9) (Zou *et al.* 2013). Although, Muhmmad *et al.* (2001) found that the strain H7N3 was inactivated at a pH of 10 in 24 hours.

A personal communication with David Swayne, a USDA expert in avian viruses stated that: “The USA DHS a few years ago confiscated some of these “100 year old eggs” smuggled from China and they were negative for AI and NDV. In discussions with the virologist, they would be surprise if any virus would survive since they are kept at the high pH for about 1 year; so you have both temperature and time, along with the pH change. We thought they were of very low risk” (MPI 2009b).

As all parts of the egg will be exposed to NaOH and a pH of greater than 10 for approximately 3 weeks, avian influenza is not identified as a hazard.

Avian paramyxovirus

The Food and Agricultural Organisation (FAO 2001) recommends that a 2% solution of NaOH is effective against inactivating viruses from the family Paramyxoviridae.

Commercially prepared 100 year old eggs are exposed to a brine solution containing a concentration of approximately 4.2% NaOH.

Avian paramyxoviruses are not identified as a hazard.

Duck enteritis virus

Duck enteritis virus (DEV) is reduced in titre when exposed to a pH of 10 for 6 hours and viral inactivation is greater at a pH of 10.5 and rapid at pH 11 (Sandu and Metwally 2008). Significant viral inactivation will be expected to occur as eggs are stored for approximately 3 weeks at a pH of greater than 10. DEV is not identified as a hazard.

⁶ Infectious bronchitis virus (IBV): there is no evidence for the transmission or isolation of IBV in eggs (MPI 2009a).

Goose and Muscovy duck parvoviruses

Parvoviruses can be inactivated after being exposed to a 2% solution of NaOH for ten minutes (Food and Agricultural Organisation 2001). Due to commercially produced alkalised eggs being exposed for 3 weeks to a brine solution containing a concentration of NaOH of approximately 4.2%, parvoviruses are not identified as a hazard.

Group 1 adenoviruses (Angara disease)

During manufacturing eggs are exposed to a pH of greater than 10 for a minimum of 3 weeks. Adenoviruses have been assessed as stable at a pH of 3 to 9 (Hess 2013). The exposure of eggs for 3 weeks to a highly alkaline environment will likely inactivate this group of viruses. Group 1 adenoviruses are not identified as a hazard.

Muscovy duck reovirus

Avian reoviruses are stable between pH 3 and pH 9 (Jones 2000). The Food and Agricultural Organisation (2001) recommends that exposure for 10 minutes to a 2% solution of NaOH is effective against inactivating reoviruses. As outlined in the commodity definition alkalised eggs are exposed to a higher concentration of NaOH for a minimum of three weeks, therefore Muscovy duck reovirus is not identified as a hazard.

Mycoplasmosis (*Mycoplasma iowae*)

Eterpi *et al.* (2010) tested the decontamination efficacy of alkaline cleaners containing NaOH as the active ingredient on mycoplasma species dried on a stainless steel surface and found that complete inactivation occurred at a solution concentration of 0.8%. As eggs will be exposed to NaOH at a higher concentration (approximately 4.2% in commercially processed eggs) for a prolonged period of time, *M. iowae* is not identified as a hazard.

Exotic *Salmonella* spp.

Salmonella spp. will not grow at a pH of above 9.5 (New Zealand Food Standards 2013) and the strain *S. Newport* has been found to be inactivated at pH 11 in cow manure (Toth *et al.* 2012). During the alkalisation process egg albumen reaches a pH of greater than 11 and the yolk greater than 10.

The Taiwanese National Animal Industrial Foundation conducted monitoring for *Salmonella* spp. in alkalised duck eggs in retail markets in 2003 and 2004 with all results being negative (Department of Agriculture, Fisheries and Forestry 2007).

The combination of high pH and length of processing means that exotic *Salmonella* spp. are not identified as a hazard.

2.3.3 DISEASES/AGENTS NOT TRANSMITTED BY THE ORAL ROUTE

The next step is to assess if the remaining pathogens can be transmitted orally. If transmission does not occur by this route then the pathogen is not identified as a hazard.

Ornithobacteriosis

Ornithobacteriosis (*Ornithobacterium rhinotracheale*): there is no evidence for the spread of this bacterium other than by the respiratory route (MPI 2013). This organism is not identified as a preliminary hazard.

2.3.4 SUMMARY OF PRELIMINARY HAZARDS

Exotic agents associated with eggs	Inactivated by processing conditions	Transmitted orally	Further assessment needed?
Avian influenza	Yes (MPI 2009a)	N/A	No
Avian paramyxovirus (Newcastle disease virus)	Yes (FAO 2001)	N/A	No
Duck enteritis virus	Yes (Sandu and Metwally 2008)	N/A	No
Goose and Muscovy duck parvoviruses	Yes (FAO 2001)	N/A	No
Group 1 adenoviruses	Yes (Hess 2013)	N/A	No
Muscovy duck reovirus	Yes (FAO 2001)	N/A	No
<i>Mycoplasma iowae</i>	Yes (Eterpi <i>et al.</i> 2010)	N/A	No
<i>Ornithobacterium rhinotracheale</i>	No	No (MPI 2013)	No
<i>Salmonella</i> spp.	Yes (New Zealand Food Standard 2013)	N/A	No

2.3.5 PRELIMINARY HAZARD IDENTIFICATION CONCLUSION

As all identified exotic agents are either inactivated by the processing conditions or not transmissible by the oral route they are not identified as hazards and require no further assessment.

3. Drinks containing eggs

3.1 INTRODUCTION

Currently, commercially manufactured beverages containing eggs are imported into New Zealand under clause 7.19 of the Ediproic import health standard which states that the commodity must be:

- commercially prepared and packaged, and;
- in its original sealed packaging on arrival.

These current measures are not based on an import risk analysis so the Animal Imports team has asked for an assessment of the biosecurity risks associated with all imported drinks that contain egg.

3.2 COMMODITY DEFINITION

There are two types of drink containing chicken eggs that are likely to be imported into New Zealand, eggnog and the alcoholic drink advocaat.

All imported drinks will be commercially prepared and packaged. Reflecting international practise, the commodities considered are defined as follows:

Eggnog - containing greater than 1% egg yolk⁷, pasteurised to 69 °C for 30 minutes, 80 °C for 25 seconds or 83 °C for 15 seconds⁸.

Advocaat - containing greater than 14% alcohol content and made with fresh unpasteurised egg yolk. Advocaat is made with high quality fresh commercial eggs and the yolks are first separated from the albumen and then mixed with sugar. This mixture is then slowly added to the spirit of choice which is usually brandy. To ensure that the ingredients do not separate, a high-pressure homogenization step is included. Finally, the liqueur is left to settle (Gonzalez-Sanjose 2014). European law requires that every litre of advocaat must contain a minimum of 150 grams of sugar, 140 grams of egg yolk, and the alcohol content must be at least 14% (Gonzalez-Sanjose 2014).

3.3 PRELIMINARY HAZARD LIST

The first step in the risk analysis process is the identification of agents of concern and the collation of these into a preliminary hazard list of pathogens that may be associated with the commodity under consideration. Following this, the pathogens are assessed to see if the processing conditions used to manufacture the commodity can inactivate any of these pathogens. Lastly, it is assessed if oral transmission of the pathogen can occur.

⁷ <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm?fr=131.170>

⁸ <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm?fr=1240.61>

3.3.1 DISEASES/AGENTS EXOTIC TO NEW ZEALAND THAT ARE LIKELY TO BE ASSOCIATED WITH EGGS

A previously completed import risk analysis assessing the biosecurity risk of egg powder (MPI 2008a), identified pathogens that are exotic to New Zealand and likely to be associated with chicken eggs.

- Avian influenza viruses (Orthomyxoviridae)
- Avian leukosis/sarcoma (Retroviridae)⁹
- Avian paramyxovirus type 1 (Newcastle disease), 2 and 3¹⁰
- Campylobacteriosis (*Campylobacter jejuni* and others)¹¹
- *Chlamydophila psittaci*¹²
- *Escherichia coli*¹³
- Group 1 adenovirus infection (Angara disease)
- Infectious bronchitis (Coronaviridae)¹⁴
- Mycoplasmosis (*Mycoplasma iowae*)
- *Ornithobacterium rhinotracheale*
- *Salmonella* Arizonae
- *Salmonella* Enteritidis
- *Salmonella* Gallinarum-Pullorum
- *Salmonella* Typhimurium DT104

3.3.2 DISEASES/AGENTS INACTIVATED BY PROCESSING CONDITIONS

Mycoplasmosis (Mycoplasma iowae)

Mycoplasma iowae: *Mycoplasma* spp. are considered to be unstable when exposed to a liquid media and die rapidly (MPI 2011).

3.3.3 DISEASES/AGENTS NOT TRANSMITTED BY THE ORAL ROUTE

Ornithobacteriosis (Ornithobacterium rhinotracheale)

There is no evidence for the spread of this bacterium other than by the respiratory route (MPI 2013). This organism is not identified as a hazard.

^{9, 11, 12, 13} Although exotic strains of this organism was previously identified as a hazard (MPI 2008), a subsequent assessment has concluded that these exotic strains are no more virulent than those present in New Zealand (MPI 2009a), so no further assessment is required here.

¹⁰ Avian paramyxovirus type 2 and 3 are no longer considered pathogenic and transmission in eggs has not been demonstrated (Australian Department of Agriculture, Fisheries and Forestry 2013).

¹⁴ Infectious bronchitis virus (IBV): there is no evidence for the transmission or isolation of IBV in eggs (MPI 2009a).

3.3.4 SUMMARY OF PRELIMINARY HAZARDS

Exotic agents associated with eggs	Inactivated by processing conditions	Transmitted orally	Further assessment needed?
Avian influenza	No	Yes	Yes
Avian paramyxovirus (Newcastle disease virus)	No	Yes	Yes
Group 1 adenoviruses	No	Yes	Yes
<i>Mycoplasma iowae</i>	Yes (MPI 2011)	N/A	No
<i>Ornithobacterium rhinotracheale</i>	No	No (MPI 2013)	No
<i>Salmonella</i> spp.	No	Yes	Yes

3.3.5 PRELIMINARY HAZARD IDENTIFICATION CONCLUSION

The exotic agents that are identified as requiring further assessment are exotic avian influenza virus, Newcastle disease virus, group 1 adenoviruses and exotic *Salmonella* spp.

3.4 GROUP 1 AVIAN ADENOVIRUSES

3.4.1 HAZARD IDENTIFICATION

Aetiological agent

Angara disease (also known as hydropericardium syndrome) is caused by the serotype Fowl adenovirus 4 (FAdV-4) which is one of the 12 serotypes belonging to the species Fowl aviadenovirus C (Hess 2000; International Committee on Taxonomy of Viruses 2013a).

The roles of other group 1 adenoviruses within the genus *Aviadenovirus*, with the exception of quail bronchitis are not well defined (McFerran and Adair 2003). Chickens have been experimentally infected with quail bronchitis virus but natural infection has not been documented (Jack *et al.* 1987).

OIE list

Angara disease is not listed.

New Zealand status

Angara disease has never been identified in New Zealand.

Epidemiology

Angara disease was first recognised in broiler chickens from Angara Goth, Pakistan in 1988. The disease has been subsequently indentified in Iraq, Kuwait, India, Mexico, Ecuador, Peru, Chile, South and Central America, Slovakia, Greece, Russia and Japan (see Hafez 2011; Kataria *et al.* 2013).

Angara disease was originally found to be infecting broiler chickens and they appear to be most susceptible to infection, although layer chickens can also be infected (Asthana *et al.* 2013; Kataria *et al.* 2013). The duration of disease is 7 to 15 days and mortality can be as high as 80% (McFerran and Adair 2003; Asthana *et al.* 2013). Angara disease is most common in broiler breeds aged 3 to 6 weeks and in layer breeds aged 10 to 20 weeks. Outbreaks of Angara disease have also been recorded in quails, pigeons, and wild black kites (Asthana *et al.* 2013).

Clinical signs of disease are difficult to observe before mortalities occur due to the acute nature of infection (Kataria *et al.* 2013). The occurrence of hydropericardium and the identification of basophilic intranuclear inclusions in the liver are the main ways by which infection is detected (McFerran and Adair 2003).

Angara disease is contagious and vertical transmission of FAdV-4 occurs (Kataria *et al.* 2013). Horizontal transmission is also important and all excretions can contain FAdV-4 with the highest titre found in faeces (McFerran and Adair 2003). Survival of adenoviruses when exposed to heat is variable and inactivation has occurred at 56 °C after 30 minutes. Although, some strains have survived at 60 °C or 70 °C for 30 minutes (McFerran and Adair 2003). FAdV-4 may survive after being heated to 60 °C for 30 minutes or 50 °C for 1 hour, although it is may be inactivated following heating at 60 °C for 1 hour, 80 °C for 10 minutes, or 100 °C for 5 minutes (Afzal *et al.* 1991).

Hazard identification conclusion

Angara disease is not recognised as being present in New Zealand and FAdV-4 is considered exotic. FAdV-4 is vertically transmitted and has been isolated from eggs and is identified as a hazard in drinks containing eggs.

3.4.2 RISK ASSESSMENT

Entry assessment

FAdV-4 has been shown to infect layer chickens and their eggs can also become infected. The excretion of FAdV-4 normally occurs 3 weeks after infection and peaks at 5 to 9 weeks and remains at 70% after 14 weeks. Additionally, there is evidence that a second period of virus excretion occurs around peak egg production. This reactivation is thought to occur due to the stress associated with egg laying or the increase in sex hormones. This results in maximum transmission to the next generation (McFerran and Adair 2003).

The processing steps involved in making drinks containing egg can consist of pasteurisation (e.g. eggnog) or high-pressure homogenisation (e.g. advocaat) and these treatments would be likely to reduce the titre of virus in the commodity. For any single bottle of advocaat the likelihood of there being a significant titre of virus in the commodity is considered to be very low as infected egg yolk/s containing viable virus would be diluted by uninfected yolks and the other ingredients.

FAdV-4 has been shown to be heat resistant and the heat treatments as described in the commodity definition are unlikely to inactivate this virus.

FAdV-4 is also been found to survive after exposure to 50% alcohol solution.

The likelihood of entry is assessed as low.

Exposure assessment

Angara disease could only infect birds in New Zealand if they were exposed to infected egg and consumed enough of the commodity to receive an oral infectious dose.

Drinks are intended for human consumption so, for exposure to occur, susceptible species would require access to waste. It would be reasonable to assume that most waste would be disposed of through drains or sewers so exposure would be unlikely. Alternatively, unopened or partly consumed bottles may be disposed of as landfill, where exposure would also be unlikely.

The likelihood of exposure is assessed as negligible.

Risk estimation

Since the exposure assessment is negligible, the risk is assessed as negligible. FAdV-4 is assessed not to be a risk.

3.5 AVIAN INFLUENZA VIRUS

3.5.1 HAZARD IDENTIFICATION

Aetiological agent

Avian influenza (AI) viruses belong to the family *Orthomyxoviridae* and genus *Influenzavirus* A. Influenza A viruses are classified into subtypes on the basis of their haemagglutinin (H) and neuraminidase (N) antigens. Presently, there are 16 H subtypes (H1-H16) and 9 N subtypes (N1-N9) with two new subtypes currently being proposed (H17, H18) (OIE 2014a). AI virus strains are commonly separated into highly pathogenic strains (HPAI) and low pathogenic strains (LPAI) based on their pathogenicity in chickens.

OIE list

Notifiable AI (NAI) viruses are listed by the OIE. All H5 and H7 strains and any AI virus strains with a pathogenicity index above that described in Chapter 10.4 of the *Terrestrial Animal Health Code* must be notified to the OIE (OIE 2014b).

New Zealand status

Exotic AI viruses are listed as unwanted notifiable organisms (Unwanted Organism Register 2014). In New Zealand AI viruses have been isolated from healthy Mallard ducks and paradise ducks (subtype: H1, H1N3, H2, H4, H4N6, H5N1, H5N2, H6N4, H7, H10, H11 and H11N3). For the NAI viruses the H5N2 subtype was found to be non-pathogenic and the H5N1 subtype was identified as LPAI (Austin and Hinshaw 1984; Stanislawek 1992; Stanislawek *et al.* 2002; Tana *et al.* 2007; MPI 2008b).

Epidemiology

The majority of the information in this section is from Swayne *et al.* (2013) unless otherwise referenced.

AI viruses have a worldwide distribution and have been shown to infect a large number of bird species. The majority of strains have been isolated from waterfowl from the order *Anseriformes* (ducks and geese) and *Charadriiformes* (shorebirds, gulls, terns, auks) and they are considered the natural reservoir for all AI viruses. Most AI infections in wild birds produce no clinical disease with the exception of the H5N1 HPAI strain (Guangdong lineage). Chickens are not considered to be the natural reservoir for these viruses.

Transmission of AI viruses occurs mainly through the inhalation or ingestion of respiratory secretions or faeces from an infected bird, with a less common route being the consumption of infected tissue either through cannibalism or predation. The likelihood of chickens being exposed to infection is influenced by the type of animal husbandry system they are reared in. For example, chickens that are raised in a free-range husbandry system are at a higher risk of infection due to the increased chance of being exposed to infected wild birds compared to an indoor husbandry system where the likelihood of exposure will be much less.

In chickens, morbidity and mortality rates are dependent on the strain of the virus, age of the host, environmental conditions and concurrent infections. With HPAI viruses, mortality has reached 100% in some flocks.

In infected birds virus is shed from the nares, mouth, conjunctiva and cloaca. In experimental studies AI viruses have been found to replicate and be excreted in chickens for up to 36 days. HPAI viruses have been isolated from the yolk and albumen from naturally infected hens (Cappucci *et al.* 1985; Kilany *et al.* 2010). In laying hens clinical signs can include a reduction in egg production and an increase in the number of low quality eggs (OIE 2014a)

In naturally infected chickens the incubation period ranges from 3 days to 14 days. The OIE *Code* lists the incubation period as 21 days when referring to international trade in poultry and poultry products.

AI viruses are considered to be relatively unstable in the environment and can be inactivated by heat, extremes in pH, dryness, hypertonic conditions and organic solvents (Swayne *et al.* 2013).

Hazard identification conclusion

AI viruses have been isolated from the internal contents of eggs (albumen and yolk) and as such all exotic strains are identified as a hazard in drinks containing eggs.

3.5.2 RISK ASSESSMENT

Entry assessment

HPAI virus has been recovered from the internal contents of eggs and could be associated with eggs used in drinks containing eggs.

The OIE *Code* recommends that whole eggs be treated to a core temperature of 60 °C for 188 seconds to inactivate AI viruses (OIE 2014b).

For drinks that are subject to pasteurisation such as eggnog (69 °C for 30 minutes, 80 °C for 25 seconds or 83 °C for 15 seconds) the likelihood of entry is assessed as negligible.

Hens that are infected with AI may produce fewer, poor quality eggs that may not pass quality control inspection. Despite this, a small number of infected eggs may pass quality control inspections and enter into food production. Advocaat is made using high-quality fresh eggs (Gonzalez-Sanjose 2014) and any cracked or low quality eggs would not be used. This would reduce the likelihood of infected eggs being used for advocaat manufacture.

European law requires that every litre of advocaat must contain a minimum of 150 grams of sugar, 140 grams of egg yolk and that the minimum alcohol content is 14% (Gonzalez-Sanjose 2014). Any infected egg yolk containing viable virus would be diluted within the drink. AI viruses are considered to be relatively unstable in the environment (Swayne *et al.* 2013) and during manufacture high-pressure homogenization is used to prevent separation of ingredients and this processing step would reduce the titre of virus. Furthermore, exposure of AI virus to ethanol has been shown to be highly effective in reducing the titre of AI virus on human hands (Grayson *et al.* 2009). Even though the ethanol content of advocaat is much lower (14%) compared to alcohol sanitizers (61.5%) the extended exposure time (from manufacture to importation) of at least several weeks will further reduce the titre of virus in the commodity.

Considering the above factors, the likelihood of entry of AI viruses in advocaat containing is assessed to be very low.

Exposure assessment

AI viruses could only infect birds in New Zealand if they were exposed to infected egg and consumed enough of the commodity to receive an oral infectious dose.

Drinks are intended for human consumption so, for exposure to occur, susceptible species would require access to waste. It would be reasonable to assume that most waste would be disposed of through drains or sewers so exposure would be unlikely. Alternatively, unopened or partly consumed bottles may be disposed of as landfill, where exposure would also be unlikely.

The likelihood of exposure is assessed as negligible.

Risk estimation

Since the entry assessment for drinks containing pasteurised eggs is negligible and the exposure assessment for drinks containing unpasteurised eggs is negligible, the risk is assessed as negligible. AI viruses are assessed not to be a risk.

3.6 NEWCASTLE DISEASE VIRUS

3.6.1 HAZARD IDENTIFICATION

Aetiological agent

Newcastle disease virus (NDV) belongs to the family *Paramyxoviridae*, subfamily *Paramyxovirus* and genus *Avulavirus* (International Committee on the Taxonomy of Viruses 2013b). Newcastle disease is only caused by virulent avian paramyxovirus-1 (APMV-1) strains that have an intracerebral pathogenicity index (ICPI) of >0.7, or multiple basic amino acids in the virus at the C-terminus of the F2 protein and a phenylalanine at residue 117, which is the N-terminus of the F1 protein (Miller and Koch 2013).

OIE list

Newcastle disease is an OIE listed disease.

New Zealand status

Exotic strains of APMV-1 are listed as unwanted, notifiable organisms (Unwanted Organism Register 2014).

APMV-1 has been detected from several species of birds in New Zealand although all isolates have had an ICPI of <0.2 (Pharo *et al.* 2000; Stanislawek *et al.* 2002). Virulent strains have never been isolated from New Zealand.

Epidemiology

The majority of the information in this section is from Miller and Koch (2013) unless otherwise referenced.

NDV has been described as infecting over 200 bird species and it is likely that all avian species are susceptible to infection (OIE 2012). NDV is widely distributed around the world and the disease is especially severe for poultry producers in the Middle East, Africa and Asia.

NDV strains display significant variation in pathogenicity in chickens and strains have been grouped into five pathotypes based on the clinical signs in chickens (from OIE 2012).

1. Viscerotropic velogenic: a highly pathogenic form in which haemorrhagic intestinal lesions are frequently seen;
2. Neurotropic velogenic: a form that presents with high mortality, usually following respiratory and nervous signs;
3. Mesogenic: a form that presents with respiratory signs, occasional nervous signs, but low mortality;
4. Lentogenic or respiratory: a form that presents with mild or subclinical respiratory infection;
5. Asymptomatic: a form that usually consists of a subclinical enteric infection.

Within these groupings considerable overlap of signs may occur.

Transmission is primarily horizontal with NDV being shed in oropharyngeal secretions and faecal matter. Susceptible birds become infected by the oral route or by inhalation.

Vertical transmission of NDV has been difficult to definitively prove as chicks may have become infected through faeces associated with the egg shell or by exposure to a contaminated environment. There are a small number of cases where NDV has been isolated from embryonating chicken eggs, day-old hatchlings and dead-in-shell birds.

Prophylactic vaccines are used to reduce mortality and morbidity although they are not capable of completely preventing infection. Vaccination combined with robust biosecurity practises helps to limit the impact of NDV infection.

The incubation period for NDV is dependent on several factors such as the age and health of the host, the strain of the virus, route of exposure and dose received. Vaccinated hens that become infected with a virulent strain of NDV may lay fewer eggs from one week after infection with the fewest eggs being produced 2 to 3 weeks after infection. After this time, however, egg production will increase again. At around one month post infection deformed eggs may start being laid and will continue to be laid for the duration of the hen's life.

Humans can become infected with NDV although it is a minor zoonosis and the main symptom is mild conjunctivitis or on occasion fever (MPI 2009a). Infection is usually caused by direct contact with infected birds or carcasses, and human to human spread has not been documented (Alexander 2000).

Hazard identification conclusion

NDV is an exotic organism that has been isolated from chicken eggs. NDV is identified as a hazard in drinks containing eggs.

3.6.2 RISK ASSESSMENT

Entry assessment

NDV has been recovered from the internal contents of eggs and could be associated with drinks containing eggs.

The OIE *Code* recommends that a 10% salted yolk be treated to a core temperature of 55 °C for 2.9 minutes (176 seconds) to inactivate NDV (OIE 2014c).

For drinks that are subject to pasteurisation such as eggnog (69 °C for 30 minutes, 80 °C for 25 seconds or 83 °C for 15 seconds; see commodity definition) the likelihood of entry is assessed as negligible.

Hens that are infected with NDV may produce eggs that are used in food production, although there are several hurdles that will significantly reduce the titre of virus in advocaat. Prophylactic vaccines are used by the majority of egg producing countries and their use will reduce the quantity of virus that is shed into the environment (Miller and Koch 2013). Advocaat is made with high-quality fresh eggs (Gonzalez-Sanjose 2014) and any deformed infected eggs will be unlikely to pass inspection. Further, any viable virus that is present in

the commodity will be diluted by the other ingredients and during manufacture advocaat is subject to high-pressure homogenization and this process may further reduce the titre of virus.

The likelihood of entry of NDV in advocaat is assessed to be very low.

Exposure assessment

NDV disease could only infect birds in New Zealand if they were exposed to infected egg and consumed enough of the commodity to receive an oral infectious dose.

Drinks are intended for human consumption so, for exposure to occur, susceptible species would require access to waste. It would be reasonable to assume that most waste would be disposed of through drains or sewers so exposure would be unlikely. Alternatively, unopened or partly consumed bottles may be disposed of as landfill, where exposure would also be unlikely.

The likelihood of exposure is assessed as negligible.

Risk estimation

Since the entry assessment for drinks containing pasteurised eggs is negligible and the exposure assessment for drinks containing unpasteurised eggs is negligible, the risk is assessed as negligible. NDV is assessed not to be a risk.

3.7 EXOTIC SALMONELLA SPP.

3.7.1 HAZARD IDENTIFICATION

Aetiological agent

Salmonella spp. are gram-negative bacteria which belong to the family *Enterobacteriaceae*. There are approximately 2500 serovars in the *Salmonella* genus and most of these belong to the species *enterica* and subspecies *enteric*. For simplicity, only the genus name followed by the serovar will be written e.g. *Salmonella* Enteritidis.

Multiple different strains are present in each serovar, which can be differentiated by phage typing and identified by the notation DT and a number. The strain *Salmonella* Typhimurium DT104 is important as it has developed resistance to multiple types of antibiotics and is widely distributed around the world (MPI 2010).

OIE list

Fowl typhoid and pullorum disease (*Salmonella* Gallinarum-Pullorum) are listed by the OIE.

New Zealand status

Salmonella Typhimurium is endemic in New Zealand, although, *S. Typhimurium* DT104 has been rarely isolated from humans and, in New Zealand, it has only been recorded once in three dogs in a household in which the owner was suffering from diarrhoea after returning from overseas (Julian 2002). The annual surveillance report of notifiable and other diseases in New Zealand reported no incidences of *S. Typhimurium* DT104 infection for the period 2009-2013 (Environmental Science and Research Limited 2014). *S. Typhimurium* DT104 is classified in the category of “other exotic organism” and is an unwanted organism (Unwanted Organisms Register 2014).

Salmonella Gallinarum has never been isolated in New Zealand and *Salmonella* Pullorum was last isolated from poultry in 1985 (MPI 2009a).

S. Enteritidis phage type 4 is the second most common *S. Enteritidis* isolated from humans in New Zealand although none have been recorded from poultry (MPI 2009a).

Epidemiology

Salmonella spp. have been isolated from virtually all species of vertebrates that have been tested and this genus of bacteria can contaminate the environment for a considerable period of time (Penrith *et al.* 2004).

Transmission of *Salmonella* spp. occurs mainly by the faecal oral route. Oral infection results in the multiplication of the organism in the intestine and invasion of the intestinal mucosa, followed by replication in the intestinal lymphatic tissue. Bacterial replication may also occur in the lungs if the organism is introduced intranasally (Penrith *et al.* 2004). Vertical transmission to chicken eggs can occur through the contamination of the ovum following ovulation (Shivaprasad and Barrow 2013).

The course of infection is dependent on the infectious dose, serovar virulence and the host's age and immune response. Losses from infection with *S. Gallinarum-Pullorum* tend to be highest in

chicks 2 weeks old and losses rapidly decline at 3-4 weeks of age (Shivaprasad and Barrow 2013).

Salmonellosis is a zoonosis and can be easily transmitted from animals to humans by ingestion or inhalation (Fenwick and Collet 2004).

Hazard identification conclusion

Exotic *Salmonella* spp. have been isolated from chicken eggs, can be transmitted orally and are zoonotic. They are identified as a hazard in drinks containing eggs.

3.7.2 RISK ASSESSMENT

Entry assessment

Salmonella spp. which are a gram-negative bacteria are more sensitive to high-pressure homogenization compared to gram-positive bacteria (Kelemen and Sharpe 1979; Vachon *et al.* 2002). Diels *et al.* (2006) concludes that high-pressure homogenization kills gram-negative bacteria in an “all or nothing” physical disruption of the cell wall.

Most *Salmonella* serotypes do not survive after being heated to 60 °C for 2.9 minutes in liquid whole egg (Mitscherlich and Marth 1984).

Pasteurisation of liquid whole egg at 60 °C for 3.5 minutes is the standard treatment used to inactivate *Salmonella* spp. in the United States (Dawson *et al.* 2012).

For drinks that are subject to pasteurisation such as eggnog (69 °C for 30 minutes, 80 °C for 25 seconds or 83 °C for 15 seconds; see commodity definition) the likelihood of entry is assessed as negligible.

European law requires that every litre of advocaat must contain a minimum of 150 grams of sugar, 140 grams of egg yolk and that the minimum alcohol content is 14% (Gonzalez-Sanjose 2014). *Salmonella* Typhimurium has been found to not grow in mid-strength or full-strength beers (Menz *et al.* 2011). Further, the ethanol content of advocaat (14%) will inactivate *Salmonella* spp. as an ethanol content of 5-10% in beer is one of the major antimicrobial hurdles (Vriesekoop *et al.* 2012). Due to the high-pressure homogenization of advocaat and the presence of ethanol at a concentration of at least 14%, the likelihood of entry is assessed to be negligible.

Risk estimation

Since the entry assessment is negligible, the risk is assessed as negligible. *Salmonella* spp. is assessed not to be a risk.

4. Muscle protein powder containing egg from all countries

4.1 INTRODUCTION

Currently, muscle protein powder containing egg is commercially imported into New Zealand from all countries under clause 7.16 of the Ediproic import health standard which states that the commodity must be:

- shelf-stable;
- commercially prepared and packaged;
- homogenously mixed so that the ingredients are evenly distributed in the mixture; and,
- in its original sealed packaging on arrival.

The above measures are not based on a risk analysis so as part of the ongoing import health standard development the Animal Imports team has requested a risk assessment be completed.

4.2 COMMODITY DEFINITION

The product under consideration is commercially manufactured muscle protein powder containing hens' eggs (*Gallus gallus*) from all countries.

Egg albumen powder is the only egg ingredient in this commodity and during manufacturing it is processed at a minimum temperature of 54.4 °C for at least 7 days (MPI 2008a).

4.3 PRELIMINARY HAZARD LIST

4.3.1 DISEASES/AGENTS EXOTIC TO NEW ZEALAND THAT ARE LIKELY TO BE ASSOCIATED WITH EGGS

The egg powder import risk analysis (MPI 2008a) lists the following pathogens as exotic to New Zealand and likely to be associated with eggs:

- Arizonosis (*Salmonella Arizonae*)
- Avian influenza (Orthomyxoviridae)
- Avian leukosis/sarcoma (Retroviridae)
- Avian paramyxovirus types 2 & 3 (Paramyxoviridae)
- Campylobacteriosis (*Campylobacter jejuni* and others)
- Colibacillosis (*Escherichia coli* 0111, 0157:H7 and others)
- Fowl typhoid (*Salmonella Gallinarum*)
- Group 1 adenovirus infections (Adenoviridae)
- Infectious bronchitis (Coronaviridae)
- Mycoplasmosis (*Mycoplasma iowae*)
- Newcastle disease (Paramyxoviridae)
- Ornithobacteriosis (*Ornithobacterium rhinotracheale*)
- Paratyphoid salmonellae (*Salmonella Enteritidis* etc.)
- Psittacosis (*Chlamydophila psittaci*)
- Pullorum disease (*Salmonella Pullorum*)

4.3.2 DISEASES/AGENTS OF CONCERN INACTIVATED BY COMMERCIAL PROCESSING CONDITIONS

As the heat treatment for muscle protein powders containing egg is the same as that described in MPI (2008) (54.4 °C for at least 7 days), the conclusions from this previous risk analysis can be directly applied to this assessment.

Accordingly, all hazards except group 1 avian adenoviruses and exotic avian influenza viruses were found to be inactivated by the heat treatment. These two pathogens will be subject to further assessment.

4.4 GROUP 1 AVIAN ADENOVIRUSES

4.4.1 HAZARD IDENTIFICATION

Aetiological agent

Angara disease (also known as hydropericardium syndrome) is caused by the serotype Fowl adenovirus 4 (FAdV-4) which is one of the 12 serotypes belonging to the species Fowl aviadenovirus C (Hess 2000; International Committee on Taxonomy of Viruses 2013a).

The roles of other group 1 adenoviruses within the genus Aviadenovirus, with the exception of quail bronchitis are not well defined (McFerran and Adair 2003). Chickens have been experimentally infected with quail bronchitis but natural infection has not been documented (Jack *et al.* 1987).

OIE list

Angara disease is not listed.

New Zealand status

Angara disease has never been identified in New Zealand.

Epidemiology

Angara disease was first recognised in broiler chickens from Angara Goth, Pakistan in 1988. The disease has been subsequently indentified in Iraq, Kuwait, India, Mexico, Ecuador, Peru, Chile, South and Central America, Slovakia, Greece, Russia and Japan (see Hafez 2011; Kataria *et al.* 2013).

Angara disease was originally described in broiler chickens and they appear to be most susceptible to infection, although layer chickens can also be infected (Asthana *et al.* 2013; Kataria *et al.* 2013). The duration of disease is 7 to 15 days and mortality can be as high as 80% (Asthana *et al.* 2013; McFerran and Adair 2003). Angara disease is most common in broiler breeds aged 3 to 6 weeks and in layer breeds aged 10 to 20 weeks. Outbreaks of Angara disease have also been recorded in quails, pigeons, and wild black kites (Asthana *et al.* 2013).

Clinical signs of disease are difficult to observe before mortalities occur due to the acute nature of infection (Kataria *et al.* 2013). The occurrence of hydropericardium and the identification of basophilic intranuclear inclusions in the liver are the main ways by which infection is detected (McFerran and Adair 2003).

Angara disease is contagious and vertical transmission of FAdV-4 occurs (Kataria *et al.* 2013). Horizontal transmission is also important and all excretions can contain FAdV-4 with the highest titre found in faeces (McFerran and Adair 2003). Survival of adenoviruses when exposed to heat is variable and inactivation has occurred at 56 °C after 30 minutes. Although, some strains have survived at 60 °C or 70 °C for 30 minutes (McFerran and Adair 2003). FAdV-4 may survive after being heated to 60 °C for 30 minutes or 50 °C for 1 hour, although it may be inactivated following heating at 60 °C for 1 hour, 80 °C for 10 minutes, or 100 °C for 5 minutes (Afzal *et al.* 1991).

Hazard identification conclusion

Angara disease is not recognised as being present in New Zealand and FAdV-4 is considered exotic. FAdV-4 is vertically transmitted and has been isolated from eggs and is identified as a hazard in muscle protein powders containing egg.

4.4.2 RISK ASSESSMENT

Entry assessment

FAdV-4 has been shown to infect layer chickens and their eggs can also become infected. The excretion of FAdV-4 normally occurs 3 weeks after infection and peaks at 5 to 9 weeks and remains at 70% after 14 weeks. Additionally, there is evidence that a second period of virus excretion occurs around peak egg production. This reactivation is thought to occur due to the stress associated with egg laying or the increase in sex hormones. This results in maximum transmission to the next generation (McFerran and Adair 2003).

FAdV-4 has been shown to be heat resistant and the heat treatments as described in the commodity definition are unlikely to inactivate this virus.

The likelihood of entry is assessed as non-negligible.

Exposure assessment

Angara disease could only infect birds in New Zealand if they were exposed to commodities containing egg.

Muscle protein powders that contain egg are a niche product used by those in the health and fitness community. It is of high value (~\$43 per 1 kilogram), is shelf stable and due to these reasons it would be expected that there would be very little wastage. The likelihood of exposure is assessed to be negligible.

Risk estimation

Since the likelihood of exposure is negligible, the risk is assessed as negligible. FAdV-4 is assessed not to be a risk.

4.5 AVIAN INFLUENZA VIRUS

4.5.1 HAZARD IDENTIFICATION

Aetiological agent

Avian influenza (AI) viruses belong to the family *Orthomyxoviridae* and genus *Influenzavirus* A. Influenza A viruses are classified into subtypes on the basis of their haemagglutinin (H) and neuraminidase (N) antigens. Presently, there are 16 H subtypes (H1-H16) and 9 N subtypes (N1-N9) with two new subtypes currently being proposed (H17, H18) (OIE 2014a). AI virus strains are commonly separated into highly pathogenic strains (HPAI) and low pathogenic strains (LPAI) based on their pathogenicity in chickens.

OIE list

Notifiable AI (NAI) viruses are listed by the OIE. All H5 and H7 strains and any AI virus strains with a pathogenicity index above that described in Chapter 10.4 of the *Terrestrial Animal Health Code* must be notified to the OIE (OIE 2014b).

New Zealand status

Exotic AI viruses are listed as unwanted, notifiable organisms (Unwanted Organism Register 2014). In New Zealand AI viruses have been isolated from healthy Mallard ducks and paradise ducks (subtype: H1, H1N3, H2, H4, H4N6, H5N1, H5N2, H6N4, H7, H10, H11 and H11N3). For the NAI viruses isolated from birds in New Zealand the H5N2 subtype was found to be non-pathogenic and the H5N1 subtype was identified as LPAI (Austin and Hinshaw 1984; Stanislawek 1992; Stanislawek *et al.* 2002; Tana *et al.* 2007; MPI 2008b).

Epidemiology

The majority of the information in this section is from Swayne *et al.* (2013) unless otherwise referenced.

AI viruses have a worldwide distribution and have been shown to infect a large number of bird species. The majority of strains have been isolated from waterfowl from the order *Anseriformes* (ducks and geese) and *Charadriiformes* (shorebirds, gulls, terns, auks) and they are considered the natural reservoir for all AI viruses. Most AI infections in wild birds produce no clinical disease with the exception of the H5N1 HPAI strain (Guangdong lineage). Chickens are not considered to be the natural reservoir for these viruses.

Transmission of AI viruses occurs mainly through the inhalation or ingestion of respiratory secretions or faeces from an infected bird, with a less common route being the consumption of infected tissue either through cannibalism or predation. The likelihood of chickens being exposed to infection is influenced by the type of animal husbandry system they are reared in. For example, chickens that are reared in a free-range husbandry system are at a higher risk of infection due to the increased chance of being exposed to infected wild birds compared to an indoor husbandry system where the likelihood of exposure is much less.

In chickens, morbidity and mortality rates are dependent on the strain of the virus, age of the host, environmental conditions and concurrent infections. With HPAI viruses, mortality has reached 100% in some flocks.

In infected birds virus is shed from the nares, mouth, conjunctiva and cloaca. In experimental studies AI viruses have been found to replicate and be excreted in chickens for up to 36 days. HPAI viruses have been isolated from the yolk and albumen from naturally infected hens (Cappucci *et al.* 1985; Kilany *et al.* 2010).

In naturally infected chickens the incubation period ranges from 3 days to 14 days. The OIE *Code* lists the incubation period as 21 days when referring to international trade in poultry and poultry products.

AI viruses are considered to be relatively unstable in the environment and can be inactivated by heat, extremes in pH, dryness, hypertonic conditions and organic solvents (Swayne *et al.* 2013). Despite this, the OIE *Code* recommends that dried egg albumen be heated to 54.4 °C for 21.38 days to achieve a 7 log reduction in virus titre (OIE 2014b).

Hazard identification conclusion

AI viruses have been isolated from egg albumen from hens that were naturally infected. Egg albumen powder that is used as an ingredient in muscle protein powders is heat treated to 54.4 °C for 7 days. The OIE recommends that egg albumen powder be heated to 54.4 °C for 21.38 days to achieve viral inactivation. Because egg albumen powder is only heated to this temperature for 7 days, AI virus will likely survive commercial processing. Exotic AI viruses are identified as a hazard in the commodity.

4.5.2 RISK ASSESSMENT

Entry assessment

The incubation period for naturally infected hens is 3 to 14 days and in one experimental study 85-100% of eggs from hens infected with HPAI contained virus at 3 and 4 days post inoculation (PI) (Burgh M, unpublished data in Swayne and Beck 2004). Because it has been demonstrated that hens can lay eggs infected with AI viruses before clinical signs develop the likelihood of infected eggs being used to make muscle protein powders is assessed as non-negligible.

Exposure assessment

Avian influenza viruses could only infect birds in New Zealand if they were exposed to the commodity.

Muscle protein powders that contain egg are a niche product used by those in the health and fitness community. It is of high value (~\$43 per 1 kilogram), is shelf stable and due to these reasons it would be expected that there would be very little wastage. The likelihood of exposure is assessed to be negligible.

Risk estimation

Since the likelihood of exposure is negligible, the risk is assessed as negligible. Exotic avian influenza viruses are assessed not to be a risk.

5. Non shelf-stable food containing up to 100% egg and frozen poached eggs

5.1 INTRODUCTION

Currently, food products containing up to 20% egg are permitted for importation under the Ediproic import health standard provided the following measures are met:

The product(s) is accompanied by an original manufacturer's declaration that specifies;

- the egg ingredient is derived from a country where Angara disease is not known to occur (the following countries are accepted free of Angara disease by MPI; European Union, Australia, USA and Canada)
- the eggs have undergone a minimum heat treatment of at least 64 degrees Celsius for no less than 2.5 minutes, or 60 degrees Celsius for no less than 3.5 minutes at any stage of the manufacturing process
- the product is commercially prepared and packaged
- the product is in its original sealed packaging on arrival

The Animal Imports team has asked for a revision of the biosecurity risks associated with non shelf-stable food containing up to 100% egg and for frozen poached eggs.

5.2 COMMODITY DEFINITION

The commodities considered are non shelf-stable food containing up to 100% egg (from *Gallus gallus*) that are intended for human consumption. Examples of such items include; frozen ready-to-eat meals, chilled ready-to-eat meals, frozen poached eggs and frozen baked goods.

Frozen poached eggs will be cooked at 70 °C for 2 minutes. For all other food products containing egg they will be subjected to pasteurisation (minimum temperature of 60 °C for at least 3.5 minutes) prior to importation.

5.3 PRELIMINARY HAZARD LIST

5.3.1 NON SHELF-STABLE FOOD CONTAINING UP TO 100% EGG

The egg powder import risk analysis (IRA) (MPI 2008a) identified all the pathogens that are exotic to New Zealand and transmitted in hens' eggs. The next step was to evaluate whether pasteurisation (60 °C for 3.5 minutes) inactivated any of these pathogens. The egg powder IRA commodity definition described the same pasteurisation regime as defined here for non shelf-stable food. This means that the conclusions from this previous risk assessment can be directly applied to all non shelf-stable food that has been subject to pasteurisation. Accordingly, the sole hazard for non shelf-stable food containing up to 100% egg is Angara disease (hydropericardium syndrome; FAdV-4).

5.3.2 FROZEN POACHED EGGS

Frozen poached eggs are heat treated to 70 °C for 2 minutes which is a time and temperature combination that was not assessed in the egg powder IRA. Because of this all pathogens previously identified as exotic and associated with hens' eggs (MPI 2008a) will be re-assessed here.

The egg powder IRA (MPI 2008) lists the following pathogens as exotic to New Zealand and transmitted in the contents of hens' eggs or present on the outside of the shell:

- Avian influenza (Orthomyxoviridae)
- Avian leukosis/sarcoma (Retroviridae)¹⁵
- Avian paramyxovirus types 1 (Newcastle disease), 2 & 3¹⁶
- Campylobacteriosis (*Campylobacter jejuni* and others)¹⁷
- *Chlamydophila psittaci*¹⁸
- *Escherichia coli*¹⁹
- Group 1 adenovirus infections (Angara disease)
- Infectious bronchitis (Coronaviridae)²⁰
- Mycoplasmosis (*Mycoplasma iowae*)
- *Ornithobacterium rhinotracheale*
- *Salmonella* Arizonae
- *Salmonella* Enteritidis
- *Salmonella* Gallinarum
- *Salmonella* Pullorum

5.3.3 DISEASES/AGENTS INACTIVATED BY PROCESSING CONDITIONS

Avian influenza virus

Avian influenza viruses are heat sensitive and the OIE Code recommends that whole eggs be heated to a core temperature of 60 °C for 3.1 minutes (188 seconds) to achieve a 7-log reduction in virus titre (OIE 2014b).

Frozen poached eggs will be heat treated to 70 °C for 2 minutes (120 seconds) prior to importation and this treatment will be expected to inactivate this group of viruses.

Avian paramyxovirus (Newcastle disease virus)

Newcastle disease virus (NDV) is sensitive to heat and the OIE Code recommends that whole eggs be heated to a core temperature of either 55 °C, 57 °C or 59 °C for 42 minutes (2521 seconds), 26.2 minutes (1596 seconds) or 11.2 minutes (674 seconds) respectively. These time temperature combinations will achieve a 7-log reduction in virus titre (OIE 2014c).

^{15,17,18,19} Although exotic strains of this organism was previously identified as a hazard (MPI 2008), a subsequent assessment has concluded that these exotic strains are no more virulent than those present in New Zealand (MPI 2009), so no further assessment is required here.

¹⁶ Avian paramyxovirus type 2 and 3 are no longer considered pathogenic and transmission in eggs has not been demonstrated (Australian Department of Agriculture, Fisheries and Forestry 2013).

²⁰ Infectious bronchitis virus (IBV): there is no evidence for the transmission or isolation of IBV in eggs (MPI 2009a).

Swayne and Beck (2004) assessed inactivation rates of three different NDV strains exposed to 63 °C. The time taken for a log reduction in titre was <18 to <20 seconds. Due to the increased viral inactivation observed in NDV when exposed to higher temperatures the heat treatment applied to poached eggs of 70 °C for 2 minutes will inactivate NDV and it is not identified as a hazard in the commodity.

***Salmonella* spp.**

Silva and Gibbs (2012) have provided minimum processing times for meat and poultry food products and heating to 70 °C for 91 seconds will provide a 7 log reduction in the most thermally resistant *Salmonella* spp. (serotype: Senftenberg). As frozen poached eggs will be cooked at 70 °C for 120 seconds inactivation of all exotic *Salmonella* spp. will occur and they are not identified as hazard in this commodity.

5.3.4 DISEASES/AGENTS NOT TRANSMITTED BY THE ORAL ROUTE

The next step is to assess if the remaining pathogen can be transmitted orally. If transmission does not occur by this route then the pathogen is not identified as a preliminary hazard.

Ornithobacteriosis

Ornithobacteriosis (*Ornithobacterium rhinotracheale*): there is no evidence for the spread of this bacterium other than by the respiratory route (MPI 2013). This organism is not assessed as a preliminary hazard.

5.3.5 PRELIMINARY HAZARD IDENTIFICATION CONCLUSION

For non shelf-stable food that contain egg and are subject to pasteurisation the sole organism requiring further assessment is group 1 avian adenovirus.

For frozen poached eggs, group 1 avian adenoviruses and *Mycoplasma iowae* require further assessment.

5.4 GROUP 1 AVIAN ADENOVIRUSES

5.4.1 HAZARD IDENTIFICATION

Aetiological agent

Angara disease (also known as hydropericardium syndrome) is caused by the serotype Fowl adenovirus 4 (FAdV-4) which is one of the 12 serotypes belonging to the species Fowl aviadenovirus C (Hess 2000; International Committee on Taxonomy of Viruses 2013a).

The roles of other group 1 adenoviruses within the genus Aviadenovirus, with the exception of quail bronchitis virus are not well defined (McFerran and Adair 2003). Chickens have been experimentally infected with quail bronchitis virus but natural infection has not been documented (Jack *et al.* 1987).

OIE list

Angara disease is not listed.

New Zealand status

Angara disease has never been identified in New Zealand.

Epidemiology

Angara disease was first recognised in broiler chickens from Angara Goth, Pakistan in 1988. The disease has been subsequently indentified in Iraq, Kuwait, India, Mexico, Ecuador, Peru, Chile, South and Central America, Slovakia, Greece, Russia and Japan (see Hafez 2011; Kataria *et al.* 2013).

Angara disease was originally found to be infecting broiler chickens and they appear to be most susceptible to infection, although layer chickens can also be infected (Asthana *et al.* 2013; Kataria *et al.* 2013). The duration of disease is 7 to 15 days and mortality can be as high as 80% (Asthana *et al.* 2013; McFerran and Adair 2003). Angara disease is most common in broiler breeds aged 3 to 6 weeks and in layer breeds aged 10 to 20 weeks. Outbreaks of Angara disease have also been recorded in quails, pigeons, and wild black kites (Asthana *et al.* 2013).

Clinical signs of disease are difficult to observe before mortalities occur due to the acute nature of infection (Kataria *et al.* 2013). The occurrence of hydropericardium and the identification of basophilic intranuclear inclusions in the liver are the main ways by which infection is detected (McFerran and Adair 2003).

Angara disease is contagious and vertical transmission of FAdV-4 occurs (Kataria *et al.* 2013). Horizontal transmission is also important and all excretions can contain FAdV-4 with the highest titre found in faeces (McFerran and Adair 2003). Survival of adenoviruses when exposed to heat is variable and inactivation has occurred at 56 °C after 30 minutes. Although, some strains have survived at 60 °C or 70 °C for 30 minutes (McFerran and Adair 2003). FAdV-4 may survive after being heated to 60 °C for 30 minutes or 50 °C for 1 hour, although it is may be inactivated following heating at 60 °C for 1 hour, 80 °C for 10 minutes, or 100 °C for 5 minutes (Afzal *et al.* 1991).

Hazard identification conclusion

Angara disease is not recognised as being present in New Zealand and FAdV-4 is considered exotic. FAdV-4 is vertically transmitted and has been isolated from eggs and is identified as a hazard in non shelf stable products containing up to 100% eggs and frozen poached eggs.

5.4.2 RISK ASSESSMENT

Entry assessment

FAdV-4 has been shown to infect layer chickens and their eggs can also become infected. The excretion of FAdV-4 normally occurs 3 weeks after infection and peaks at 5 to 9 weeks and remains at 70% after 14 weeks. Additionally, there is evidence that a second period of virus excretion occurs around peak egg production. This reactivation is thought to occur due to the stress associated with egg laying or the increase in sex hormones. This results in maximum transmission to the next generation (McFerran and Adair 2003).

FAdV-4 has been shown to be heat resistant and the heat treatments as described in the commodity definition are unlikely to inactivate this virus.

The likelihood of entry is assessed as non-negligible.

Exposure assessment

Angara disease could only infect birds in New Zealand if they were exposed to commodities containing egg.

The majority of non-shelf stable food products containing up to 100% egg would be cooked for a second time prior to consumption. This would further reduce the infectivity of any FAdV-4 in the commodity and any food waste produced would likely present a negligible risk of exposure.

In contrast, imported food products containing egg that are not exposed to a second heat treatment and are discarded could be consumed by backyard chickens if composted or susceptible wild birds if scavenged from a compost or refuse station.

Pigeons are a host of Angara disease (McFerran and Adair 2003) and broiler chicken have been shown to become infected after consuming liver suspensions from infected pigeons (MPI 2008a).

The likelihood of exposure to backyard chickens and wild avian species is assessed to be low.

Consequence assessment

Infected wild avian species could horizontally transmit this virus to domestic poultry on farms that have inadequate biosecurity, although the likelihood of this occurring is low (MPI 2008a). The mortality rate in broiler flocks infected with FAdV-4 can be as high as 80%. (Asthana *et al.* 2013).

Once Angara disease established it would be difficult and costly to eradicate as it is contagious with horizontal and vertical transmission aiding the spread of this disease (MPI 2008a).

Angara disease and other group 1 adenoviruses pose no risk to human health.

A number of avian species are susceptible to infection by group 1 adenoviruses (McFerran and Adair 2003) and it is likely that some native species would be susceptible to infection if Angara disease became established in New Zealand.

The consequence of introducing Angara disease into New Zealand is assessed as non-negligible.

Risk estimation

Since the entry, exposure and consequence assessments are non-negligible, the risk is assessed as non-negligible. FAdV-4 is assessed to be a risk.

5.4.3 RISK MANAGEMENT

Options

The risk management measures from MPI (2008) could also be considered for non shelf-stable food containing up to 100% egg and frozen poached eggs and are listed below:

- Assurance could be required that eggs used have been derived from flocks in countries or geographic regions where Angara disease has not been recognised.
- The source flock could be tested to ensure freedom from FAdV-4. Diagnostic tests for avian adenovirus using PCR are available.
- Studies of liver homogenate extracts have shown that heat treatment of 60°C for greater than one hour destroys FAdV-4 infectivity. Further heat treatment if practical could be used to inactivate any virus present.

5.5 MYCOPLASMA IOWAE

5.5.1 HAZARD IDENTIFICATION

Aetiological agent

Mycoplasma iowae is a very small prokaryote bacterium belonging to the family Mycoplasmataceae and class Mollicutes. This bacterium is devoid of a cell wall and colonies grow in a characteristic “fried egg” shape (Kleven 2003).

OIE list

Mycoplasma iowae is not an OIE listed disease.

New Zealand status

Mycoplasma iowae has not been reported in New Zealand and is listed as an unwanted organism (Unwanted Organism Register 2014).

Epidemiology

Turkeys are the natural host of *M. iowae* and infection has also been found to occur in chickens and several species of wild birds in the United Kingdom (Al-Ankari and Bradbury 1996).

The main routes of transmission in turkeys are venereal and transovarial. Horizontal transmission has been demonstrated but in young birds that are not yet sexually mature the prevalence is low and rate of spread slow (Bradbury and Kleven 2003).

The role of *M. iowae* as a pathogen is uncertain, with even the same author expressing differing views. For example Bradbury (2001) included *M. iowae* as one of four economically important mycoplasmas, while in a review article on *M. iowae* by Al-Ankari and Bradbury (1996) the conclusion was that “there is insufficient data to reach any conclusions about the economic significance, if any, of *M. iowae* infections in turkeys, or in chicks or chick embryos”, and Bradbury (pers.comm.²¹) commented “...we have never used PCR to look for this Mycoplasma (*M. iowae*) because it is no longer considered important enough to be of interest”.

Eggs from naturally infected turkeys have a decrease in hatchability of 2-5% (Bradbury and Kleven 2003). Experimental infection with *M. iowae* has caused mortality in turkey and chicken embryos and also airsacculitis and leg abnormalities in inoculated individuals (Al-Ankari and Bradbury 1996). A study of naturally infected chickens by Bencina *et al.* (1991) identified the presence of *M. iowae* and a number of other mycoplasma species in a flock that was of mixed ages and had not been depopulated for more than 15 years. In comparison *M. iowae* was not isolated from three farms that contained single-age flocks that were depopulated annually.

M. iowae has not been identified as occurring in New Zealand, although it is considered likely that it has been introduced with turkey hatching eggs and has remained undetected in the absence of a targeted surveillance programme (MPI 2006).

²¹ Bradbury JM. Personal communication to Bruce Simpson 23rd July 2004.

Hazard identification conclusion

Vertical transmission of *M. iowae* has been demonstrated in turkeys and it is assumed this can occur in chickens. *M. iowae* is identified as a hazard in frozen poached chicken eggs.

5.5.2 RISK ASSESSMENT

Entry assessment

M. iowae is only likely to be present in eggs from chicken flocks that are unhusked, exposed to other avian species and that contain multiple age groups (MPI 2009a). Frozen poached eggs will be sourced from commercial flocks that are well managed and the likelihood that *M. iowae* will be present in these eggs is assessed to be negligible.

Risk estimation

Since, the risk of entry is assessed to be negligible, the risk is estimated to be negligible. *Mycoplasma iowae* is assessed not to be a risk.

6. References

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