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The likelihood of avian influenza and avian orthoavulavirus-1 being present in eggs that are made into egg powder

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Foreword

This report was funded by the Department of Agriculture, Water and the Environment. It was produced by a team led by the Commonwealth Scientific and Industrial Research Organisation (CSIRO).

The terms of reference for the task were specifically to:

- provide a literature review assessing reports of avian influenza and avian orthoavulavirus -1 detection in eggs, and
- to determine the likelihood of these viruses being present in the eggs that are made into egg powder.

In addition to this, it has been ascertained from the literature review the steps in which inactivation of these viruses could occur during the process of turning eggs into egg powder.

Contents

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Executive summary

The importation of egg powders from overseas countries is one potential pathway for the introduction of exotic avian influenza virus (AIV) and orthoavulavirus-1 (AOAV-1) into Australia. A literature review was conducted to assess reports of these viruses being detected in eggs, and to determine the likelihood of these viruses being present in eggs that are made into egg powder.

Overall, AIV and AOAV-1 presence in eggs that are turned into egg powder is very unlikely and the likelihood of these viruses being present in imported powdered egg products into Australia is negligible. The key reasons for this are:

- Flocks infected with AIV or AOAV-1 that show clinical signs are routinely destroyed globally, and their eggs destroyed or pasteurised if used.
- Neither virus readily enters the internal contents of the egg
- Virus replication can only occur in fertilised eggs, however eggs that are processed into powder are unfertilised
- The standard process of turning eggs into egg powder involving pasteurisation and spray drying is sufficient to inactivate AIV and thermolabile AOAV-1, should they be present. However, the pasteurisation evidence for thermostable AOAV-1 is less conclusive.
- Australia's additional import conditions of final heating of the powdered products are a further step that would contribute to virus inactivation, should they be present

1 Background

1.1 Avian influenza virus

Avian influenza virus (AIV), an Influenza A virus of the family Orthomyxoviridae, causes avian influenza (AI) infection. There are two pathotypes; low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI). HPAI in particular has caused substantial losses to poultry industries globally, including in Australia (Swayne, 2016). Avian viruses are classified into subtypes based on surface haemagglutinin (H) and neuraminidase (N) glycoproteins, of which there are currently 16 H subtypes (H1–H16) and 9 N (N1-N9) (OIE, 2018b; Swayne, 2016). Mutations from LPAI to HPAI have only been associated with subtypes H5 and H7. AIVs classified as HPAI and H5 and H7 LPAI viruses with amino acid sequences similar to HPAI viruses are notifiable to the World Organisation for Animal Health (OIE) (OIE, 2018b). Australian-lineage LPAI viruses naturally circulate in Australian wild birds (Grillo et al., 2015). However, Australia is considered free of LPAI and HPAI infection in domestic poultry, where an AI specific disease strategy manual exists to effectively eradicate the disease if detected in Australian poultry (Animal Health Australia, 2011).

1.2 Avian orthoavulavirus-1

Newcastle disease (ND) is caused by virulent strains of avian orthoavulavirus type 1 (AOAV-1, formerly avian paramyxovirus type 1), of the genus Orthoavulavirus and family Paramyxoviridae. Like HPAI, ND has caused substantial losses to poultry industries globally, including in Australia (OIE, 2018a). Avirulent Australian-lineage strains of AOAV-1 are endemic in Australian poultry and wild birds. Few avirulent strains have the potential to become virulent over time, but include those that share a cleavage site sequence with virulent strains (Animal Health Australia, 2014; OIE, 2018a). Virulent strains of AOAV-1 are notifiable to the OIE (OIE, 2018a). Exotic AOAV-1, particularly virulent strains, are of concern to Australia. Australia has developed a Newcastle disease management plan to facilitate decision making if an exotic and/or virulent strain of AOAV-1 is detected (Animal Health Australia, 2020).

1.3 Appropriate level of protection (ALOP)

Under the Sanitary and Phytosanitary Measures (SPS) agreement, World Trade Organization (WTO) members, including Australia, are entitled to maintain an appropriate level of protection (ALOP) to protect life or health when importing goods within their territory. However, measures must only be applied to the extent necessary to protect life or health and be based on scientific principles set by international bodies. The OIE establishes the health standards for international trade in animals and animal products (World Trade Organization, 2020). Under the SPS agreement and the OIE standards, Australia must consider the importation of poultry products from other countries if there is sufficient scientific evidence that the ALOP can be achieved. This includes countries in which exotic AI or AOAV-1 virus are endemic in poultry (DAWE, 2020).

1.4 Virus introduction through imported egg powders

Transforming whole eggs into egg powder has been a convenient and popular process for the global food industry. Egg powders have an extended shelf life, reduced transport and storage costs, and are relatively unsusceptible to bacterial growth compared to whole eggs. The transformation is most commonly done via spray drying, where whole egg liquid is finely sprayed into hot air (Lechevalier, Nau, & Jeantet, 2013).

The importation of egg powders from overseas countries in which AI or AOAV-1 viruses are endemic or occur sporadically in poultry is one potential pathway for the introduction of these viruses into Australia. This issue can be addressed in part by assessing the likelihood of these viruses being present in eggs that are made into egg powder. Additionally, assessing whether standard egg powder manufacturing processes are sufficient to inactivate such viruses should they be present provides additional insight into the level of protection offered by Australia's import conditions. A literature review has been conducted to determine this likelihood, with the findings described in the following sections.

Part I Avian influenza and avian orthoavulavirus-1 detection in eggs

2 Avian influenza

Tropism and replication of AIVs in the reproductive tract of chickens has been previously described for some AIV subtypes. This has occurred through demonstration of positive immunostaining of reproductive tissue sections via immunohistochemistry and through virus detection from ovarian and oviductal tissue samples (Sá e Silva, Rissi, Pantin-Jackwood, & Swayne, 2013; Shalaby, Slemons, & Swayne, 1994). Virus replication in ovarian tissue and in epithelial cells of the oviduct can theoretically lead to virus shedding in the yolk and albumen of eggs respectively (Sá e Silva et al., 2013).

The detection of HPAI virus inside the albumen or yolk of eggs has been documented in both naturally and experimentally infected hens. Virus was recovered at a lower frequency from eggs laid from chicken flocks not showing clinical signs compared to chickens with clinical signs during a HPAI H5N2 outbreak in Pennsylvania in 1984 (Beato, Capua, & Alexander, 2009; Cappucci et al., 1985). HPAI H5N2 virus was more readily detected in albumen samples (30%) than yolk or mixed albumen and yolk samples (20%) from five of seven naturally infected chicken flocks during this outbreak (Cappucci et al., 1985). Similarly, HPAI H5N1 virus was detected in eggshell and albumen, but not yolk in naturally infected flocks in Egypt. In this case, virus was also only detected in flocks that showed clinical signs (Kilany et al., 2010). During a HPAI H5N1 outbreak in Hong Kong in 1997, virus was detected in 3 of 30 eggshell washes from duck and goose eggs (Beato et al., 2009; Li et al., 2006).

Experimental infection of chickens with HPAI H5N2 virus led to the recovery of virus in 12 out of 14 eggs on day 3 post infection, concurrent with the onset of significant clinical signs. Virus was detected in the albumen and yolk in eleven and nine of the eggs in this experiment respectively (Beard, Brugh, & Johnson, 1984; Spickler, Trampel, & Roth, 2008). In chickens experimentally infected with HPAI H5N8 virus, virus titres were generally higher in eggshell swabs, followed by albumen then yolk. Only three of seven infected hens produced virus-containing eggs in this experiment and this only occurred on day 3 post infection (Uchida, Takemae, Tanikawa, Kanehira, & Saito, 2016). In chickens experimentally infected with HPAI H7N8 virus, virus was detected on or in 11.1% (9/81) of eggs produced, of which 44.4% (4/9) were abnormal eggs. Virus was detected on 6.2% (5/81) of eggshell swabs and in 4.2% (2/48) of mixed albumen and yolk samples where separate testing for albumen and yolk was not conducted. Overall, HPAI H5N8 virus detection in normal eggs was at 7% in this experiment (Stephens, Spackman, & Pantin-Jackwood, 2020).

Less is known about LPAI virus presence in eggs, but available information suggests that the rate of detection of LPAI virus in eggs is lower than that of HPAI virus. LPAI virus was detected in the internal contents of 2 of 120 eggs of clinically unaffected chickens in the vicinity of a HPAI H5N2 outbreak in Pennsylvania (Cappucci et al., 1985). In chickens experimentally infected with LPAI H7N8 virus, no abnormal eggs were produced and virus was only detected on or in 6.4% (15/234) of the eggs; 5.6% (13/232) on the shells and 1.5% (2/137) in mixed albumen and yolk samples (Stephens et al., 2020). LPAI viruses were not detected in any egg samples from 120 eggs collected during a LPAI H7N2 outbreak (Lu et al., 2004). Viruses were also not detected in any egg samples from chickens

experimentally infected intra-tracheally and intravenously with LPAI H4N8 virus. However, AIV was recovered from the ovarian and oviduct tissue of these hens in this experiment (Shalaby et al., 1994).

Detection of AIVs is generally at a higher rate on eggshells compared to the internal contents of the eggs. Contamination of the eggshell most likely occurs after the egg is formed or laid, rather than from transovarial or oviductal transmission. As the eggshell is exposed to the external environment, there are more opportunities for contamination to occur. Faecal/cloacal contamination of the eggshell can occur when AIV replicates in the digestive tract and is shed through the faeces, thereby potentially contaminating the eggshell during the laying process. Similarly, AIV in dust, dander or feathers can lead to surface contamination of the egg after the egg is laid (Spickler et al., 2008; Stephens et al., 2020). It is possible for the internal contents of eggs to be contaminated through viral penetration of AIV through the eggshell (Beato et al., 2009). Microbial contamination of eggs depends on environmental conditions such as hygiene, temperature, and surface that eggs are laid onto. Cracked eggs are more likely to become internally contaminated (Mayes & Takeballi, 1983).

3 Avian orthoavulavirus-1

There are few reports on the detection of AOAV-1 in table eggs. However, detection and distribution of AOAV-1 in the ovarian follicles and oviduct of the hen has been previously reported (Bwala, Clift, Duncan, Bisschop, & Oludayo, 2012; Sá e Silva, Susta, Moresco, & Swayne, 2016). Transmission of AOAV-1 from the reproductive tract of the hen to the internal contents of the egg is therefore theoretically possible.

Like AIV, AOAV-1 can also be transmitted through faecal contamination of the egg or via other environmental contamination when the egg is laid (D. J. Alexander, 1988; Capua, Scacchia, Toscani, & Caporale, 1993). Experimentally, a study evaluating the amount of AOAV-1 penetration through eggshell surfaces yielded low amounts of virus in both uncracked and cracked eggs. Penetration did not go further than the cuticle and was observed in 3.34% and 1.67% of uncracked eggs after 30 minutes and 48 hours of exposure of the eggshell to the AOAV-1 respectively. Penetration to the outer shell membrane occurred in 10% of cracked eggs after 24 hours of exposure to AOAV-1. Virus penetration did not go further than the outer shell membrane in any of the uncracked and cracked eggs (Williams & Dillard, 1968).

One study demonstrated that ND vaccination of chickens decreases AOAV-1 contamination in eggs. Unvaccinated and vaccinated chickens were experimentally infected with virulent AOAV-1. Virus was isolated at day 3 post-challenge in 6/10 eggshell swabs, 3/10 albumen samples and 2/10 yolk samples in the non-vaccinated group. In birds vaccinated against ND once, virus was isolated from three eggshell swabs at day 3 post-challenge and for those vaccinated twice virus was isolated on one swab at the same time period. No virus was detected in the internal contents of the eggs for both of these vaccinated groups (Sá e Silva et al., 2016). Virulent AOAV-1 was isolated from embryonated eggs that were derived from a naturally infected commercial breeder chicken flock. The detection of virulent AOAV-1 in live embryos was unexpected as ND usually leads to the cessation of egg production, abnormal eggs and/or dead embryos (Capua et al., 1993).

Part II The likelihood of virus presence in the eggs that are made into egg powder

4 Eggs used for egg powder and likelihood of virus replication

Both AI and AOAV-1 viruses need living cells to propagate. In fertilised eggs, these viruses can propagate in the embryo and chorioallantoic membrane layers if the temperature inside the egg is optimal (Oldoni, Brown, King, Samal, & Seal, 2005; Spackman & Killian, 2014). There is no virus amplification in unfertilised eggs (Gonzales et al., 2018).

The vast majority of eggs produced for human consumption, including those processed into egg powder, are unfertilised. There is no economic value of commercial farms rearing male chickens, and so these farms are structured to only have female chickens (Stadelman, 1994). Chicks are sexed before being placed on farms, where males are terminated early (Phelps et al., 2003; Stadelman, 1994). In the absence of roosters, fertilisation of eggs on these farms therefore does not occur. Vent sexing is the most common method used to sex chicks and has a 95% accuracy with skilled workers. Other automated methods including those based on DNA techniques or estrogen levels have higher sexing accuracy rates (Blond, 2018; Cerit & Avanus, 2007; Phelps et al., 2003). Due to the structure of the commercial egg industry and the high sexing accuracy rates, the likelihood of a male chicken being present on a commercial farm is very low. Therefore, the likelihood of a fertilised egg which potentially allows for virus replication entering the food chain is also very low.

5 Heat treatments and inactivation of viruses in eggs

Processing of egg yolk, whole egg, or egg white into powder requires drying of the raw product. For egg yolk and whole egg, a pasteurisation step of the raw product is included prior to drying (Lechevalier et al., 2013). Pasteurisation of egg products is predominantly performed to reduce microbial contamination of food safety pathogens, particularly *Salmonella*, from eggs (James, Lechevalier, & Ketteringham, 2002). Pasteurisation of egg white is generally performed following drying. Pasteurisation temperature and time recommendations for both egg yolk and whole egg range from 60°C for 3.5 minutes in the USA to 65°C for 5-6 minutes in Europe. Pasteurisation of egg white powder for 55–65°C for 3–5 days, otherwise known as dry heating, is commonly used to also improve egg white powders' foaming and gelling properties. Further improvement of such properties has been demonstrated at higher temperature and time conditions of 80°C for 5–10 days (Lechevalier et al., 2013).

There are various drying processes that are used to turn egg products into powder, the main process of which is spray drying. Spray drying involves finely spraying the liquid egg product into a stream of hot air followed by quick cooling to minimise heat damage to the product. Air temperatures during spray drying rarely exceed 180°C, 165°C and 145°C for egg white, whole egg and egg yolk, respectively (Lechevalier et al., 2013). There are no studies which look at virus inactivation that could occur through spray drying specifically, however, virus inactivation is possible given the high temperature and desiccation that the products are subjected to during the process.

The Australian Government's Biosecurity import conditions for egg yolk, whole egg, or egg white powder state that all products must be spray dried. Although there is no explicit import condition on the pasteurisation of egg or egg yolk prior to processing into powder, spray dried egg or egg yolk powder must be dry heated to a minimum core temperature of not less than 70°C for at least 120 minutes. Similarly, spray dried egg white must be heated in its final packaging for a minimum of either 70°C for at least 7 days, or 62°C for at least 10 days (DAWE, 2021). Figure 1 provides a flowchart of the egg powder production process including the Australian import conditions and which steps would lead to virus inactivation, defined as at least a 5 or 6-log reduction of virus.

A study by Swayne and Beck (2004) demonstrated that 8 or less hours was sufficient to achieve a 1-log reduction for each of the three AOAV-1 viruses in dried egg white when incubated at 55^{o}C. By extrapolation of this result, a 6-log reduction in AOAV-1 virus in dried egg white at 55^{o}C is likely to be achieved in 48 hours. The study also indicated that one industry pasteurisation protocol for dried egg white (54.4°C for 7 to 10 days) was not effective for inactivating HPAI virus. However, a follow up study indicated that this pasteurisation protocol was adequate, and that the failure to inactivate in the previous study was likely due to the moisture content of the experimental dried egg white which was neither controlled nor measured. Higher moisture content favours virus survival (Thomas & Swayne, 2009). Differences in product preparation between the two studies must also be noted. In the previous study, dried egg whites were rehydrated in sterile water followed by virus inoculation, rather than virus inoculation of the liquid egg white occurring prior to drying in the later study, of which the latter more closely replicates natural virus contamination in eggs (Swayne &

Beck, 2004; Thomas & Swayne, 2009). The follow up study demonstrated a 7-log reduction in HPAI virus in dried egg white in 2.6 days at 54.4°C, with shorter times required to achieve similar levels of virus inactivation when the samples were incubated at higher temperatures. The study concluded that the pasteurisation guidelines in place for commercially available spray dried egg white are effective at inactivating HPAI virus with a large safety margin (Thomas & Swayne, 2009). The OIE Terrestrial Animal Health Code states suitable time and temperatures for a 7-log reduction of AIV and AOAV-1 in dried egg white. For AIV, the minimum time and temperature is 73.2 hours at 51.7°C, and for AOAV-1 it states 50.4 hours at 57°C (OIE, 2019b, 2019a).

The thermostability of different strains of AOAV-1 has been studied previously. In general, virulent forms of AOAV-1 are more thermostable than avirulent forms. Studies have shown that most AOAV-1 strains lose infectivity when exposed to 50-55°C for 30 minutes in allantoic fluid. At 56°C, most AOAV-1 strains had a 2 to 3-log infectivity reduction within 10 minutes in allantoic fluid. However, some virulent strains required at least 10 minutes (Lomniozi, 1975; Wambura, Meers, Spradbrow, & Meers, 2006). A study of chicken meat homogenate artificially infected with AOAV-1 (strain Herts 33/56) showed that the times needed for 1-log reduction of virus at 65°C, 70°C, 74°C and 80°C was 120, 82, 40 and 29 seconds respectively (Dennis J. Alexander & Manvell, 2004). The same study also assessed the reduction of virus in infected amnio-allantoic fluid and demonstrated a 5.6-log reduction after 180 seconds at 65°C and complete inactivation at 360 seconds. At 80°C, a 7.1-log reduction was achieved after 40 seconds. The study noted that the difference in results between the chicken meat homogenate and the amnio-allantoic fluid matrices was a result of a protective effect from the greater concentration of protein and organic matter in the meat homogenate (Dennis J. Alexander & Manvell, 2004). From the analysis by Alexander and Manvell (2004), it can be assumed that incubation of liquid egg products at 65^{o}C for 6 mins should be sufficient to achieve at least a 6-log reduction in thermostable AOAV-1 virus titre, at least for the strain tested.

Swayne and Beck (2004) demonstrated that the time taken for a 1-log reduction for the AIV strains and one of the three AOAV-1 strains in whole egg at 59^{o}C was <23 seconds. The other two AOAV-1 strains were more thermostable and had 1-log reduction times of 57.6 and 96.3 seconds at this temperature. However, the time taken for a 1-log reduction in all of the viruses tested in whole egg at 61°C and 63°C was similar at <20 seconds (Swayne & Beck, 2004). In contrast, an earlier study demonstrated a 1-log reduction of thermostable AOAV-1 in liquid whole egg in 38 seconds at 64.4°C (Gough, 1973). However, this earlier study was performed using thicker-walled and larger-volume glass vials which would have contributed to longer heating up and cooling down periods upon transition in and out of the water bath used. The more recent study involved a more precise method using thin-walled and smaller-volume plastic tubes which were incubated in a thermocycler (D. J. Alexander & Chettle, 1998; Gough, 1973). If it is accepted that the more recent study by Swayne and Beck (2004) is more accurate, it can be assumed that incubation of whole egg at 61^{o}C for 2 mins should be sufficient to achieve at least a 6-log reduction in AIV or AOAV-1 virus titre, at least for the strains tested. It is acknowledged that one of the common pasteurisation guidelines of 60^{o}C for 3.5 mins is at a lower temperature and higher time period compared to this study, but there is insufficient evidence to precisely state whether these conditions would lead to a 6-log reduction in thermostable AOAV-1 in whole egg.

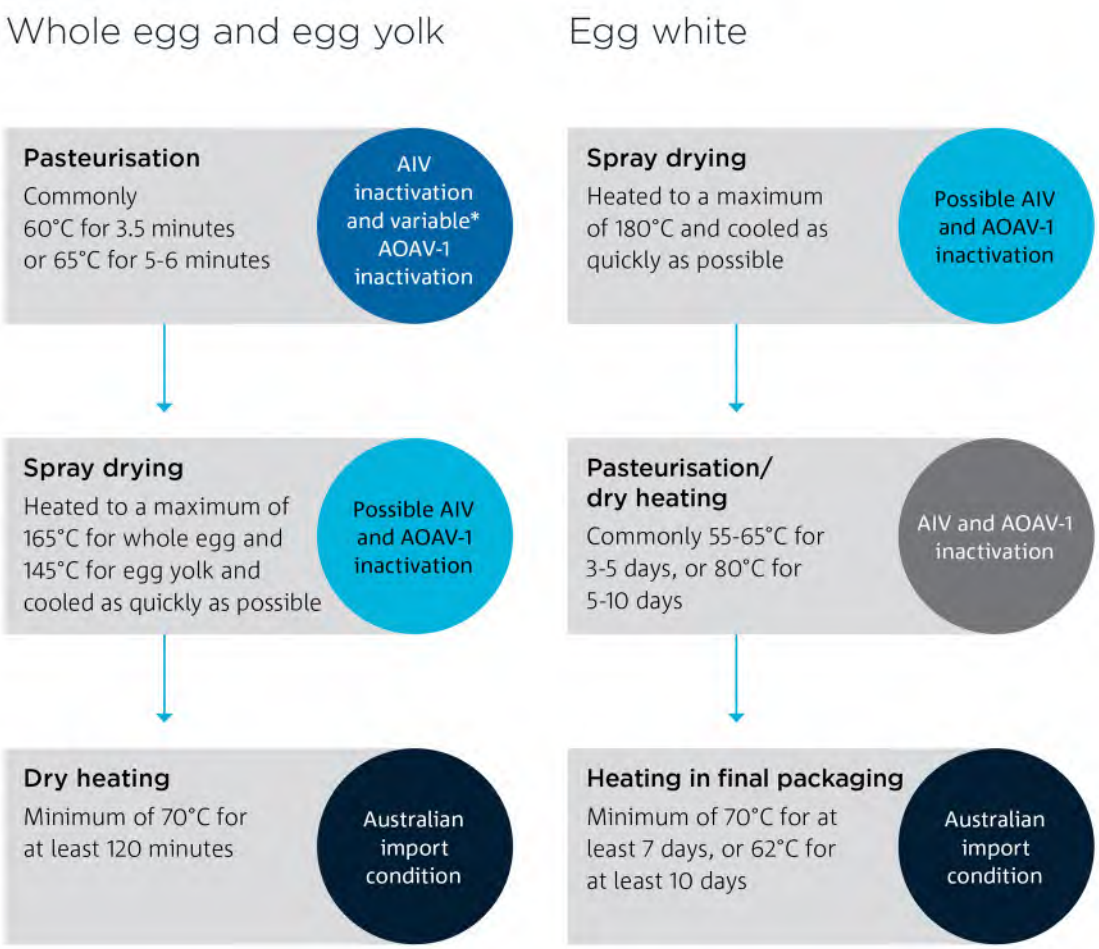

Figure 1. Stages during the egg powder production process when virus inactivation would most likely occur, including the final product processed in accordance with Australian import conditions.

* As discussed in section 5, there is insufficient evidence to state that 60°C for 3.5 minutes will lead to inactivation of some thermostable strains of AOAV-1. However, 65°C for 5-6 minutes does appear to be sufficient.

AIV is avian influenza virus and AOAV-1 is avian orthoavulavirus-1.

Inactivation refers to at least a 5 or 6 log reduction of virus.

6 The likelihood of virus presence in imported egg powder

The findings of this literature review are similar to those of the European Food Safety Authority (EFSA), which conducted an assessment of LPAI virus transmission via raw poultry meat and raw table eggs (Gonzales et al., 2018). In the assessment by the EFSA, the presence of LPAI virus in table eggs was very unlikely to negligible, and the combined probability of exposure and subsequent LPAI virus infection was negligible for commercial poultry and humans.

In this review, the chance of AIV or AOAV-1 presence in eggs to be turned into egg powder for importation in Australia is very unlikely. The chance of virus replication is negligible as table eggs are unfertilised. For AIV, higher virus detection in eggs was associated with HPAI infected flocks and from chickens showing clinical signs but the rate of virus detection in these studies was still relatively low. It must also be noted that the rapid destruction of birds infected with HPAI or ND is a common control strategy globally, where eggs are either destroyed or pasteurised if used (OIE, 2019a, 2019b). Cessation of egg laying and the production of abnormal eggs are also common clinical signs in flocks infected with these viruses, and so eggs may not even be available for collection (Capua et al., 1993). Virus presence was found to be greater on eggshells, followed by albumen and then yolk, indicating that internal shedding of the virus occurs less commonly than external contamination of eggs.

As shown above, it is very unlikely that AIV or AOAV-1 viruses will be present in the eggs made into egg powders. Notwithstanding this, the standard industry processes of turning eggs into egg powder are sufficient to inactivate AIV and thermolabile AOAV-1 should they be present in the eggs made into egg powder. However, the pasteurisation evidence for thermostable AOAV-1 is less conclusive (Dennis J. Alexander & Manvell, 2004; Swayne & Beck, 2004). The spray drying process required in the condition improves the chance of virus inactivation if present due to subjecting the products to intense heat and desiccation (Lechevalier et al., 2013; Thomas & Swayne, 2009). Although there is no import condition on the pasteurisation of egg product prior to processing into powder, it is commonly performed for whole egg and egg yolk. Similarly, dry heating/ pasteurisation of egg white powder is commonly performed to improve foaming and gelling properties of the product.

In addition to the standard industry processes, Australia's import conditions include a requirement for dry heating spray dried egg or egg yolk powder and heating egg white powder in its final packaging. These further steps would likely contribute to virus inactivation, should they be present.

Given that AIV and AOAV-1 presence in eggs is very unlikely, that standard industry processes are likely sufficient to inactivate these viruses if present (noting that there is less conclusive evidence for thermostable AOAV-1), and that Australia's import conditions provide additional risk management beyond this, the overall likelihood of these viruses being present in imported powdered egg products into Australia is negligible.

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