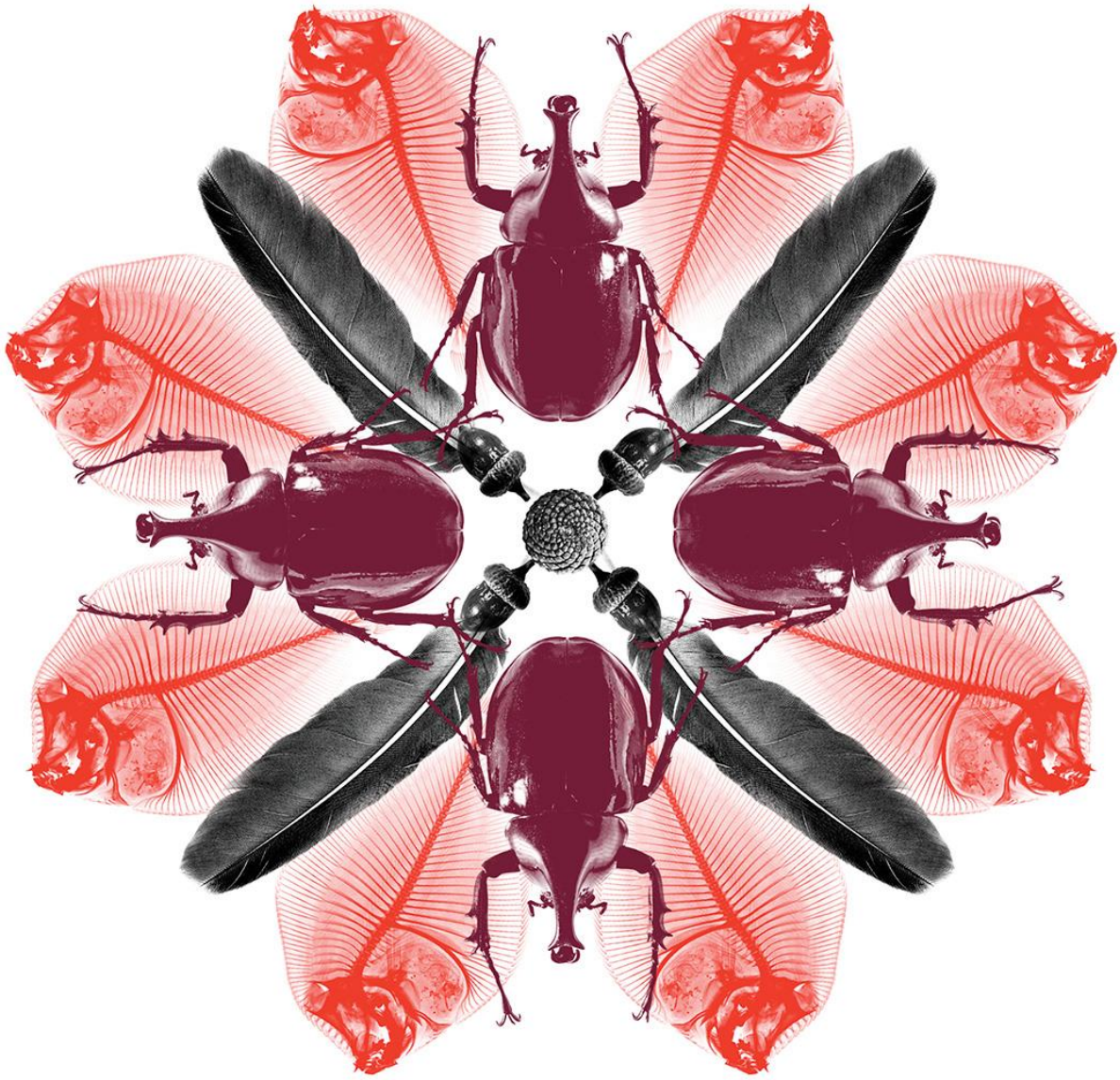
Australian Government
Department of Agriculture

Assessment of the scientific aspects of high pressure processing as an equivalent risk management measure to the heat treatments in the *Generic import risk analysis for chicken meat: final import risk analysis report* (2008)

Final report

August 2019



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Contents

Acronyms and abbreviations

Term or abbreviation	Definition
AI/AIV	avian influenza/avian influenza virus
AC-ELISA	antigen-capture enzyme-linked immunosorbent assay
CAM	chorioallantoic membrane
CFU	colony forming unit
CSIRO	Commonwealth Scientific and Industrial Research Organisation
EID	egg infectious dose
HPP	high pressure processing
IBD/IBDV	infectious bursal disease/infectious bursal disease virus
IRA	*Generic import risk analysis for chicken meat: final import risk analysis report* (2008)
ND/NDV	Newcastle disease/Newcastle disease virus
OIE	World Organisation for Animal Health (Office International des Epizooties)
RT-PCR	reverse-transcription polymerase chain reaction
SPF	specific-pathogen-free
TCID	tissue culture infectious dose
w/w	weight for weight, the proportion of a substance within a mixture, as measured by weight

Summary

The Department of Agriculture (the department) has prepared this final report of its assessment of the scientific aspects of high pressure processing (HPP) as an equivalent risk management measure to the heat treatments in the *Generic import risk analysis for chicken meat: final import risk analysis report* (2008) (chicken meat IRA) (Biosecurity Australia 2008) in response to stakeholder interest and provision of scientific material. This final report considered stakeholder submissions on the draft report. No technical information was received that warranted revision of the assessment presented in the draft report.

Australia currently only permits the importation of chicken meat subject to the application of risk management measures as specified in the chicken meat IRA. The chicken meat IRA recommends risk management measures for nine pathogens of biosecurity concern:

- high pathogenicity avian influenza virus (AIV)
- low pathogenicity AIV
- Newcastle disease virus
- very virulent infectious bursal disease virus (IBDV)
- exotic antigenic variant strains of IBDV
- four serotypes of *Salmonella enterica* subspecies *enterica*—*S.* pullorum, *S*. gallinarum, *S*. enteritidis and multidrug-resistant strains of *S*. typhimurium.

The department has assessed using HPP treatment for chicken meat to manage the risk of these pathogens to an equivalent level. The department found that currently available scientific literature on HPP inactivation of pathogens of biosecurity concern is limited and preliminary. Studies suggest that HPP treatment is effective at inactivating a range of microorganisms to varying degrees, but the efficacy depends on several factors.

At this stage, the body of evidence for HPP is insufficient to allow the department to develop import conditions that manage the risk for all pathogens of concern to an appropriate level in the variety of chicken meat products that fall under the chicken meat IRA definition. Consequently, this final report concludes that HPP of chicken meat is not considered to be a suitable alternative risk management measure to the heat treatments in the chicken meat IRA.

This final report contains details of the assessment, key findings and final recommendations.

1 Introduction

1.1 Australia's import conditions for chicken meat

The import of chicken meat into Australia is subject to Australia's *Biosecurity Act 2015* and the application of risk management measures as specified in the Department of Agriculture's (the department's) *Generic import risk analysis report for chicken meat: final report 2008* (chicken meat IRA) (Biosecurity Australia 2008). The chicken meat IRA recommends risk management measures for nine pathogens:

- high pathogenicity avian influenza virus (AIV)
- low pathogenicity AIV
- Newcastle disease virus (NDV)
- very virulent infectious bursal disease virus (IBDV)
- exotic antigenic variant strains of IBDV
- four serotypes of *Salmonella enterica* subspecies *enterica*— *S.* pullorum, *S*. gallinarum, *S*. enteritidis and multidrug-resistant strains of *S*. typhimurium.

Section 1.7 and Appendix A outline the current certification requirements for these pathogens.

The department is responsible for managing the biosecurity risk of imported goods under the *Biosecurity Act 2015*. It can decide to review or amend an import policy if alternative risk management measures demonstrate an equivalent level of biosecurity protection, or if new, peer-reviewed scientific information becomes available. In line with the *Biosecurity Act 2015*, the department assesses the level of biosecurity risk associated with a good proposed for import to determine if it achieves Australia's Appropriate Level of Protection (ALOP). Australia's ALOP is defined in the *Biosecurity Act 2015* as providing a high level of sanitary and phytosanitary protection aimed at reducing biosecurity risks to a very low level, but not to zero.

1.2 High pressure processing

High pressure processing (HPP) treatment is a nonthermal processing alternative to heat treatment to inactivate pathogenic and spoilage microorganisms in food. The pressure used in HPP treatment is expressed in megapascals (MPa); 1 MPa is equal to 1,000,000 pascals, which is the International System for Units derived unit of pressure. Food is sealed in flexible, water-resistant packaging and subjected to high levels of hydrostatic pressure, commonly between 300 and 800 MPa, for seconds to minutes at temperatures ranging from refrigeration temperatures (approximately 1–4 °C) up to 45 °C. The process is applied to both liquids and solid foods with a high moisture content, and has the benefit of having little effect on food texture, appearance (including colour), taste and nutritional value. The commercial use of HPP is increasing, particularly for processing of foods where heat treatment would adversely affect the product quality. The technology is commonly used for food safety purposes and to extend the shelf life of products such as juices; fruits, vegetables and salads; sliced ham, turkey or chicken meat; and other ready-to-eat products.

A strong body of scientific literature supports using HPP to inactivate food safety–related pathogens and spoilage microorganisms, but the scientific evidence demonstrating the use of HPP to inactivate pathogenic agents of animal biosecurity concern in food products is limited.

The technology has not been used for this purpose in Australia or overseas, based on available information.

1.3 Request to assess equivalence

In 2017 the department commenced an assessment to determine if HPP of chicken meat is equivalent to the biosecurity risk management measures in the chicken meat IRA. This assessment was undertaken in response to long-standing stakeholder interest in having HPP assessed as an equivalent risk management measure. In this context, the department was provided with unpublished information and a peer-reviewed scientific paper by Buckow et al. 2017 that was funded by a stakeholder. The scientific paper notes that the funding agent had no role in the collection, analysis and interpretation of data, nor in the submission of the article for publication. All publication costs were borne by CSIRO.

1.4 Stakeholder engagement

The department invited stakeholder comments on the scientific aspects of HPP as an equivalent risk management measure to the heat treatments in the chicken meat IRA (see Biosecurity Advices 2017-08 and 2017-13).

Ten submissions were received from individuals, Australian poultry industry members, a state government and an overseas government competent authority. Of these, one submission supported HPP as an equivalent risk management measure to the heat treatments in the chicken meat IRA.

Most submissions discussed one or more of the following points:

- The findings of Buckow et al. (2017) and other available literature could not be extrapolated to other muscle meat or commercial chicken products.
- The study by Buckow et al. (2017) was inadequate in scope, size and/or duration; or the hypotheses, method and/or findings were not sound.
- There is a lack of evidence about the efficacy of HPP treatment for many pathogens of concern in both Buckow et al. (2017) and other available literature.
- There is a need to investigate factors affecting the efficacy of HPP—including temperature, pH, water activity, fat and oil content (type and dispersal), and salt and sugar concentrations—in more detail regarding commercial chicken meat products.

Some submissions included various pieces of scientific evidence (outlined in references provided by stakeholders). The department assessed the information provided in the submissions and the associated scientific evidence, and gathered additional scientific evidence (outlined in references sought by the department), where appropriate, to determine whether HPP of chicken meat is equivalent to the heat treatment requirements in the chicken meat IRA.

The department released a draft report for a 60-day public consultation period on 17 April 2019 (see Biosecurity Advice 2019-A02). Following stakeholder feedback, the consultation period was extended to 17 July 2019 (see Biosecurity Advice 2019-A03). Four submissions were received during the consultation period; three indicated support and none provided new technical information relevant to the assessment outlined in the report. Therefore, the department determined that revision of the assessment presented in the draft report was not required. The department then completed this final report, which will be published on the department's

website along with a notice advising stakeholders of the release. The department will also notify the registered stakeholders and the World Trade Organisation (WTO) Secretariat about the release of the final report. Publication of the final report represents the end of the assessment process.

1.5 The assessment

Buckow et al. (2017) investigated HPP as a way to inactivate avian viral pathogens in chicken meat homogenate. Inactivation studies were conducted for AIV, NDV and IBDV (see Box 1 for the paper's abstract).

The department assessed the findings of Buckow et al. (2017) and information provided in stakeholder submissions and relevant scientific information, to determine whether HPP treatment of chicken meat could achieve equivalent inactivation of pathogens of biosecurity concern to the heat treatments described in the chicken meat IRA.

For this assessment, the definition of chicken meat as outlined in the chicken meat IRA was applied: 'the whole or part of the carcass of any domestic chicken (*Gallus gallus*) (but excluding the head, feathers, and all offal other than the liver, heart, gizzard, neck and feet)' (Biosecurity Australia 2008).

Pathogen inactivation is usually measured in $\log_{10}$ reductions in infectious titre[1]. The chicken meat IRA outlines that 'a product will be considered to present a "negligible" risk of introduction of a pathogenic agent, if it undergoes processing capable of achieving a titre reduction of at least 6 logs (that is, 10^6) before export to Australia' (Biosecurity Australia 2008). This 6 $\log_{10}$ reduction formed the basis for the equivalence assessment for pathogen inactivation.

The department has compiled its key findings and final recommendations in this document. Chapter 2 (the main assessment) is split into four sections. The first three sections address factors relevant to HPP efficacy in inactivation of microorganisms surrounding product composition (Section 2.1), HPP parameters (Section 2.2) and pathogens of biosecurity concern (Section 2.3). As the Buckow et al. (2017) paper was provided as evidence for the equivalence of HPP treatment for inactivation of pathogens of biosecurity concern, parts of sections 2.1–2.3 relate to this paper. The final section specifically addresses the findings of the paper and the comments raised on the paper in the stakeholder consultation process (Section 2.4).

[1] Log, short for logarithm, is a power to which a base number can be raised to produce a given number. A reduction of 1 $\log_{10}$ reduces pathogen titres by 90 per cent (10 fold), 2 $\log_{10}$ reduces pathogen titres by 99 per cent (100 fold), 3 $\log_{10}$ reduces pathogen titres by 99.9 per cent (1,000 fold), and so on.

Box 1: Abstract from Buckow et al. (2017)

High pressure processing was investigated as a means to inactivate avian viral pathogens in chicken meat homogenate. Preliminary studies were conducted on eight viruses: avian influenza virus (AIV), Newcastle disease virus (NDV) and six strains of infectious bursal disease virus (IBDV). Application of 600 MPa at room temperature for up to 2 min resulted in substantial decline of virus infectivity of all strains of IBDV, AIV and NDV in chicken meat homogenate. An inactivation kinetic of IBDV-Tasik94, a very virulent strain of IBDV, showed 5 to 6 $\log_{10}$ reduction of 50% egg infectious doses (EID_{50})/0.2 ml in chicken meat homogenate within 10–15 s treatment at 600 MPa and room temperature. However, when measured in eggs, around 0.5 to 2.0 $\log_{10}$ EID_{50}/0.2 ml of infectious virus remained, even after longer treatment times of up to 2.5 min. The inactivation curve was fitted to different kinetic models and a Log-decay model described best the rapid initial decline of infectivity followed by a persistent 'tail' of resistant IBDV-Tasik94. Multiple pressure cycles at 600 MPa were not able to further reduce the titre of IBDV in chicken meat homogenate; however, these resistant IBDV particles were not infectious to chickens when challenged via the mucosal route. This study could inform policy on risk assessment of the importation of chicken meat products.

Source: Buckow et al. (2017)

1.6 External review

Because of the complexity of the assessment and the submissions received, Scientific Advisory Group (SAG) members reviewed a comprehensive preliminary assessment of HPP equivalency. The SAG members—acting as independent experts—reviewed and commented on the department's process for the assessment and whether all stakeholder submissions were appropriately considered. SAG members delivered their comments on the preliminary assessment on 28 June 2018, which were considered in the draft assessment.

The SAG members then reviewed the draft assessment and were satisfied that it contained a thorough review of the issues raised and included extensive additional consideration of scientific literature and international standards. The draft assessment set out clearly the conclusions of the department's analysis of the scientific literature and international standards. It also set out clear questions that would need to be addressed satisfactorily before HPP as an alternative risk management measure to heat treatments in the chicken meat IRA would be assessed again.

SAG members considered that the department had followed a reasonable process during its assessment and had properly considered all stakeholder submissions. As no revision of the draft assessment was required following the stakeholder consultation period, no further external review was undertaken for this final report.

1.7 Current approval and certification requirements

Appendix A details the current certification requirements for imported chicken meat. The chicken meat IRA contains additional requirements for chicken slaughter and chicken meat processing establishments.

The department has procedures in place to approve exporting countries for the purposes of exporting chicken meat to Australia, to ensure that Australia's requirements can be met. This includes an assessment of the country in relation to the following criteria:

- avian health status with respect to the nine pathogens of biosecurity concern, including any surveillance programs in place for domestic poultry or testing for export purposes
- animal health legislation
- systems for control over certification of animals and products (particularly poultry meat products)
- veterinary and laboratory services
- performance in disease notification
- biosecurity requirements (particularly for live birds and avian products)
- disease management and control programs
- general veterinary services capacity.

In-country verification activities are normally undertaken prior to approving a country to export chicken meat to Australia.

In addition, a selection of chicken slaughter and chicken meat processing establishments are assessed. For approval to be granted, the chickens must be slaughtered and the meat prepared in establishments equivalent to the requirements in Food Standards Australia New Zealand's *Primary Production and Processing Standard for Poultry Meat (Australia only)* (Standard 4.2.2) (FSANZ 2012).

2 Key findings

2.1 High pressure processing efficacy in inactivation of microorganisms: product composition

A product's composition affects how efficacious HPP treatment is in inactivating microorganisms. This includes a product's pH, fat and oil content, water activity (a_w), and salt and sugar concentrations.

2.1.1 pH

With regard to inactivation, various pathogens respond differently to HPP depending on the pH of the medium they are present in. Even small changes in pH can significantly affect the level of pathogen inactivation achieved.

The NSW Food Authority infers that, for most microbes, as the pH of a product decreases (becomes more acidic), it requires a shorter hold time under pressure to inactivate the microbes present, and vice versa (NSW Food Authority 2016). Work by Linton, McClements and Patterson (1999) on the HPP inactivation of *Escherichia coli* in orange juice found the initial pH to significantly affect the efficacy of treatment. Linton et al. demonstrated that $\log_{10}$ inactivation of *E. coli* declined as pH increased (became more alkaline), and that even a small increase in pH of 0.2 could reduce inactivation by >2 $\log_{10}$.

A study by Kingsley and Chen (2009) on the influence of pH on pressure inactivation of hepatitis A virus (HAV) found that an acidic environment significantly improved pressure inactivation of HAV at various temperatures tested. At 20 °C they found a 2.9 $\log_{10}$ difference in titres between pH 3 and pH 7. Interestingly, Kingsley and Chen (2008) found contrasting results when investigating feline calicivirus, which was more resistant at lower pH.

Considine et al. (2008) discussed how the environment around the microorganism can significantly influence HPP inactivation, and that a low pH suspending medium can render pathogens more sensitive to HPP effects. They also highlighted that pressurisation of buffers (such as those used in experimental studies) can generate extensive shifts in pH, which would render such data unrealistic in food situations.

Buckow et al. (2017) used a chicken breast meat homogenate that was 10 per cent buffer (virus suspension). Although a neutral saline was used (pH 7.2), it is unknown how the use of this buffer affected the pH under pressurisation. The authors did not report the pH range of the chicken breast meat used in the study.

The pH of chicken meat can vary between breed, line and age of the chicken, and factors such as transport, cooling and storage periods (Glamoclija, N et al. 2015). After slaughter, muscle pH declines due to glycolysis (the remaining glucose in the meat metabolises and produces lactic acid). Once this pH decline ceases, the ultimate pH of muscle is reached. Berri et al. (2005) described the effects of ante-mortem stress on muscle pH for three lines of chickens and factors that induced an increased ultimate pH in heavy-line chickens. This study found significantly higher ultimate pH in breast and thigh muscle of fast-growing chickens than in slow-growing chickens.

Ali, Kang and Joo (2008) described how chickens exposed to heat stress before slaughter showed the lowest ultimate pH and birds shackled for a longer time the highest. Soglia et al.

(2016) found that breast muscles of birds from the same flock of heavy broilers exhibited varying ultimate pH. Breast fillets affected by wooden breast and/or white striping (muscle abnormalities that are more common in heavier lines) exhibited significantly higher ultimate pH than normal breasts. Kumar and Rani (2014) studied the chemical composition of the meat from various cuts of poultry obtained from three commercial brands. For pH, they found that values ranged considerably between both brand and cut of meat:

- breast fillets ranged between 7.58 ±0.01 and 8.00 ±0.04
- breast fillets with skin ranged between 5.73 ±0.07 and 6.88 ±0.07
- thigh cuts, wings and drumsticks (bone-in) ranged between 5.77 ±0.02 and 6.91 ±0.01.

Conclusions

- pH affects the efficacy of HPP. Increasing pH is associated with a decrease in efficacy for inactivation of some pathogens, and vice versa for other pathogens.
- Small changes in pH can have significant effects on HPP efficacy for inactivating microorganisms.
- Buckow et al. (2017) did not investigate the effects of pH on inactivation.
- Commercial chicken products are likely to have variable pH.
- It is currently not possible to specify what combinations of pH (value or range) and HPP parameters (time, pressure and temperature) would be suitable to inactivate pathogens of biosecurity concern in chicken meat products.

2.1.2 Fat and oil content

The presence of fat can protect microorganisms by increasing their resistance to pressure (NSW Food Authority 2016; Rendueles et al. 2011). D'Souza, Karwe and Schaffner (2014) found that HPP was not a suitable technology to manage the microbiological safety of *Salmonella*-contaminated peanut butter—a high-fat, low a_w food.

The fat content of different cuts of chicken meat (for example, breast, thighs, bone-in/out) is variable. Jaturasitha et al. (2008) compared the fat content of breast and thigh muscle across several breeds, including two common commercial breeds—Bresse and Rhode Island Red. The breast muscle of Bresse and Rhode Island Red breeds comprised 0.76 g and 0.72 g of fat per 100 g, respectively, and the thigh muscle comprised 4.21 g and 6.04 g of fat per 100 g, respectively. Skin has substantially more fat content than both breast and thigh muscle, with modern broiler skin containing close to 50 per cent fat (Cliche et al. 2003). Fat content will also differ for bone-in products, as bone contains marrow, which has a high fat content.

Furthermore, Soglia et al. (2016) investigated breast muscles of birds from the same flock of heavy broilers and found that breast fillets affected by wooden breast and/or white striping muscle abnormalities exhibited significantly higher fat content than normal fillets. The study found that normal breast meat had a fat content of 0.87 per cent, increasing to 1.25 per cent in breast meat affected by wooden breast and 1.98 per cent in breast meat affected by both wooden breast and white striping.

Buckow et al. (2017) did not report the fat content of the chicken breast meat used in their study. They used breast meat from specific-pathogen-free (SPF) chickens obtained from SPAFAS Australia Pty Ltd (Woodend, Victoria, Australia). It is unknown whether abnormalities such as

wooden breast and/or white striping were present in the breast meat. No other cuts of meat were tested.

Conclusions

- Fat content affects the efficacy of HPP, as fat has a baroprotective effect (that is, it protects microorganisms from being inactivated due to high pressure treatment).
- Numerous factors will affect the fat content of chicken meat, including the cut of meat, inclusion of skin or bone, and presence of abnormalities.
- Buckow et al. (2017) did not investigate the impact of fat content on inactivation.
- Commercial chicken products are likely to have variable fat content.
- It is currently not possible to specify what combinations of fat content (value or range) and HPP parameters (time, pressure and temperature) would be suitable to inactivate pathogens of biosecurity concern in chicken meat products.

2.1.3 Water activity (a_w)

Water activity is the partial vapour pressure of water in a solution or substance, divided by the partial vapour pressure of pure water at the same temperature. In simpler terms, a_w can be described as water availability. When all water is available, $a_w = 1$, and when there is a total absence of 'free' water molecules, $a_w = 0$. In food science, free water molecules are those that are not bound to food molecules or otherwise interacting with solutes and surfaces, and are available for other hydration interactions.

Cheftel (1995) reported that a low water content of the medium or food undergoing pressure treatment exerts a very strong baroprotective effect. D'Souza, Karwe and Schaffner (2014) investigated the potential of HPP to inactivate *Salmonella* in peanut butter and reported that 'HPP seems to have limited effectiveness against *Salmonella* under low-water activity conditions'. They determined that HPP 'alone or with other formulation modification … is not a suitable technology to manage the microbiological safety of *Salmonella*-contaminated peanut butter' and that 'the addition of peanut oil to further lower the water activity of peanut butter further reduced the effectiveness of HPP' (D'Souza, Karwe & Schaffner 2014). When water was added to increase the a_w of the peanut butter, a steady and significant increase in $\log_{10}$ reduction was seen, as added moisture increased from 50 per cent to 90 per cent.

Oxen and Knorr (1993) demonstrated that reducing a_w from 0.96 to 0.91 markedly reduced inactivation rates in microbial suspensions measured at one atmosphere. Although reducing a_w appears to protect microbes against inactivation by HPP, 'it is expected that microbes may be sublethally injured by pressure, and recovery of sublethally injured cells can be inhibited by low a_w. Consequently, the net effect of water activity may be difficult to predict. Foods are more pressure-protective for microorganisms than buffers or microbiological media' (Institute of Food Technologists 2000). The report outlines that a_w is a critical process factor that requires careful monitoring for every batch of food treated by HPP.

In addition, 'reducing water activity (a_w) of the medium by addition of solutes such as sugars and salts at high concentrations may exert a baroprotective effect on microorganisms' (Daryaei, Yousef & Balasubramaniam 2016). The impact of salt and sugar is discussed in Section 2.1.4.

Buckow et al. (2017) did not report the a_w of the medium tested; however, it is assumed to be higher than chicken breast meat alone due to homogenisation and addition of the saline-based

virus suspension. It is therefore possible that HPP would have reduced efficacy to inactivate microorganisms in other chicken products.

Conclusions

- The a_w of the product undergoing treatment affects the efficacy of HPP.
- Buckow et al. (2017) did not investigate the impact of a_w on inactivation.
- Commercial chicken products are likely to have variable a_w.
- It is currently not possible to specify what combinations of a_w (value or range) and HPP parameters (time, pressure and temperature) would be suitable to inactivate pathogens of biosecurity concern in chicken meat products.

2.1.4 Salt and sugar concentration

The NSW Food Authority (2016) stated that presence of minerals and sugars increases microbial resistance to pressure. Cheftel (1995) also reported that high salt or sugar concentrations exert a very strong baroprotective effect. Reducing a_w by adding solutes such as sugars and salts likely contributes to this baroprotective effect.

Kingsley and Chen (2008) found that both salt (NaCl) and sucrose (table sugar) influenced the inactivation of feline calicivirus (FCV) by HPP. The amount of FCV inactivated by pressure was inversely proportional to the sucrose or NaCl concentration. For example, a sample treated without added sucrose reduced the FCV titre by 5.1 $\log_{10}$ plaque forming units (PFUs)/ml; however, titre reduction decreased to 0.9 $\log_{10}$ PFU/ml with 40 per cent sucrose. A significant difference in inactivation was observed between 0 per cent and 12 per cent NaCl, with a 5.0 $\log_{10}$ inactivation observed with no added NaCl, and a 0.7 $\log_{10}$ inactivation observed at 12 per cent NaCl. The study also found that, when both NaCl and sucrose were added, they had an additive effect on increasing the pressure resistance of FCV. Although this study was performed on virus in a suspension rather than in tissue, it shows the effect that adding salt and sucrose may have on HPP inactivation efficacy.

Raw chicken meat does not contain sugar; however, it has a variable salt (and other mineral) content. Raw skinless boneless chicken breast meat contains 45 mg of salt per 100 g, and raw skinless boneless chicken leg meat contains 96 mg of salt per 100 g (USDA 2018). Enhanced poultry products—that is, those that have been synthetically saturated (for example, marinaded or injected) with a mix of water, salt and other additives to increase juiciness and tenderness—may contain more than 380 mg of salt per 100 g. Marinaded chicken products also often contain high amounts of sugar.

Conclusions

- The presence of minerals and sugars affect the efficacy of HPP.
- Buckow et al. (2017) did not investigate the effect of mineral or sugar content of different cuts of meat, nor the effect of additives containing these.
- Commercial chicken products are likely to have variable mineral and sugar content.
- It is currently not possible to specify what combinations of mineral and/or sugar content and HPP parameters (time, pressure and temperature) would be suitable to inactivate pathogens of biosecurity concern in chicken meat products.

2.2 High-pressure processing efficacy in inactivation of microorganisms: parameters

The efficacy of HPP to inactivate microorganisms differs depending on operational parameters, including the pressure used, the temperature and the length of time a product is held under treatment. Generally, increasing pressure and/or hold time at pressure increases the degree of microbial inactivation in a product treated by HPP.

Temperature includes both the initial temperature of the HPP vessel and any changes during the treatment due to product composition. Most foods with a high moisture content have a similar heat of compression to that of water (that is, a temperature change of 3 °C per 100 MPa at an initial temperature of 25 °C), whereas foods with a high fat content have higher compression heating (for example, treatment of chicken fat results in a 4.5 °C change per 100 MPa; treatment of olive oil results in an 8.7 °C change per 100 MPa) (NSW Food Authority 2016).

The temperature of the process will affect the level of inactivation of microorganisms achieved:

> An increase in food temperature above room temperature and to a lesser extent a decrease below room temperature increases the inactivation rate of microorganisms during HPP treatment … temperature distribution must be determined in the food and reproduced each treatment cycle if temperature is an integral part of the HPP microbial inactivation process specification. (NSW Food Authority 2016)

Cheftel (1995) reported that the temperature of pressure processing influenced the resistance of microorganisms to high pressure treatment, with temperatures above 50 °C or between –30 °C and 5 °C enhancing inactivation.

In addition:

> Enveloped viruses are less stable [i.e. more susceptible] to environmental stresses than nonenveloped viruses. However, some enveloped viruses (e.g. vesicular stomatitis virus [VSV]) are much more stable than nonenveloped viruses during HPP treatment. In addition, viruses are more stable in cold environments than at room temperature. However, many viruses (e.g. human NoV, murine norovirus [MNV], and Tulane virus [TV]) are more easily inactivated by HPP at cold temperatures (e.g. 4 °C) than at ambient temperature. (Araud et al. 2015)

Buckow et al. (2017) treated samples at 600 MPa at room temperature (before compression) for a variety of treatment times. Based on the above information, any increase or decrease from room temperature is likely to have increased the inactivation rate of microorganisms present. However:

> The resource requirements for this type of research are a limitation to the scope of studies needed to fully understand the mechanisms and kinetics of virus inactivation. It is for this reason that only time, but not other parameters, notably temperature and pressure, was tested at a range of values. Nonetheless, further studies are needed to determine the effect of treatment temperature and pressure level on the kinetics of inactivation of IBDV in chicken meat. (Buckow et al. 2017)

The United States Department of Agriculture (USDA) recognises the variability in HPP effectiveness for different pathogens across products of varying composition, and the importance of verifying that appropriate critical operational parameters have been determined for products undergoing HPP antimicrobial treatment. The USDA requires that official establishments using HPP provide scientific supporting documentation to demonstrate the log reduction achieved for specific pathogens identified in the hazard analysis and the critical operational parameters necessary for the process to achieve the stated log reduction. Also, the product composition and the critical operational parameters used in the scientific supporting documentation must reflect the establishment's actual process.

USDA Food Safety and Inspection Service Directive 6120.2 (FSIS 2012) instructs inspection program personnel (IPP) to verify an establishment's intended use of HPP treatment and to perform HACCP verification tasks in official establishments that apply the HPP antimicrobial treatment as a process step. Although this directive relates to HPP treatment for food safety purposes, the principles of the verification activities are relevant for HPP treatment targeting pathogens of biosecurity concern. An extract from Directive 6120.2 is in Box 2 (non-relevant sections excluded as indicated by '…'):

Box 2: IPP VERIFICATION OF ESTABLISHMENT ACTIVITIES

…

B. When an official establishment uses HPP as support for decisions in the hazard analysis, IPP are to perform a HACCP Verification task to verify compliance with 9 CFR 417.5(a)(1) and 417.4(a)(1). Does the establishment:

…

2. Provide scientific supporting documentation to show the log reduction achieved for the specific pathogen identified in the hazard analysis and the critical operational parameters (e.g. pressure, time, temperature) necessary for the process to achieve the stated log reduction. The composition of the products and the critical operational parameters used in the scientific supporting documentation should reflect the establishment's actual process. ...

…

4. Define a process for every type of food treated. Given the variety and combinations of critical operational parameters, establishments may evaluate factors such as pH, water activity, composition, and preservatives to determine if these are critical factors for a specific food.

Source: FSIS (2012)

2.2.1 Conclusions

- Various combinations of operational parameters are possible for HPP treatment of products, and some are critical to inactivating pathogens of biosecurity concern.
- The operational parameters required will vary depending on a specific product's composition, as different factors—including those discussed previously—may influence the efficacy of treatment.
- An effective process (including critical operational parameters) should be defined for every type of product treated.
- Critical operational parameters (regarding inactivation of pathogens of biosecurity concern) have not been determined for commercial chicken products of varying composition.

2.3 High pressure processing efficacy in inactivation of microorganisms: pathogens of biosecurity concern

Numerous scientific studies demonstrate that the sensitivity to HPP of different virus species, even different strains within the same species and serotype, is variable (Araud et al. 2015; Cheftel 1995; Hirneisen et al. 2010; Kingsley 2013; Kingsley, Chen & Hoover 2004; Kingsley et al. 2007; Lou et al. 2011). The pathogens of biosecurity concern identified in the chicken meat IRA are:

- low and high pathogenicity AIVs
- NDV
- very virulent and exotic antigenic variant strains of IBDV
- *S.* pullorum, *S*. gallinarum, *S*. enteritidis and multidrug-resistant strains of *S*. typhimurium.

Section 2.2 described the effects that operational parameters have on HPP efficacy. In addition, the properties of the viruses themselves and how they interact with the parameters may introduce further complications. For example, whether the virus is enveloped or non-enveloped, and the presence of proteins that act as thermal stabilisers or increase resistance to high or low pH, can change the efficacy of HPP.

The scientific evidence available regarding the inactivation of pathogens of biosecurity concern in HPP-treated chicken meat is limited.

Isbarn et al. (2007) found that HPP at 500 MPa at 15 °C for 90 seconds achieved a greater than 5 $\log_{10}$ inactivation of AIV H7N7 Bratislava strain. However, this was in a cell culture medium supplemented with 1 per cent fetal calf serum, not chicken breast meat and is thus not comparable to commercial chicken meat products. It is likely that the log reduction would be reduced in commercial products compared with the medium used in Isbarn et al. (2007), based on the information outlined in previous sections. A chicken meat suspension was also tested; however, the chicken meat used was homogenised and then disinfected by 5.25 per cent sodium hypochlorite. Again, this is not comparable to commercial chicken meat products. Buckow et al. (2017) states 'at room temperature, AIV H7N7 is rapidly inactivated at pressures above 400 MPa. For example, 1-minute treatment at 450 MPa and 25 °C results in 7 $\log_{10}$ reduction of the virus titre in chicken meat homogenate' referencing (Isbarn et al. 2007). However, this was predicted via a mathematical model of the inactivation of the virus and provides insufficient evidence for inactivation in commercial products.

Dumard et al. (2013) inactivated human influenza virus (H3N2) by HPP for vaccine development studies. They showed that HPP inactivated this virus after treatment at 289.6 MPa at 25 °C for 3 hours. Barroso et al. (2012) used AIV (H3N8) for vaccination studies; the virus was pressurised in a high-pressure cell system for 12 hours at 300 MPa. No other information about inactivation was provided.

Different strains of NDVs are known to vary in their susceptibly to pH and heat treatments (Lomniczi 1975; Moses, Brandly & Jones 1947; Swayne & Beck 2004). However, no studies other than Buckow et al. (2017) could be identified that investigated the effect of HPP on strains of NDV.

Tian et al. (2000) investigated HPP inactivation of IBDV in bursal tissue homogenate and chicken embryo fibroblast supernatant. The study found that at 230 MPa, treatment for 10 minutes at 0 °C decreased the IBDV titre by 4.5 $\log_{10}$ tissue culture infective dose 50. At 30 minutes treatment, no further decrease in titre was observed, but by 59 minutes the titre appeared to have decreased to 0. However, the medium suspending the IBDV tested is not comparable to chicken meat and therefore the findings cannot be extrapolated further.

Tian et al. (1999) inactivated IBDV for immunogenicity studies but, similar to Tian et al. (2000), the medium suspending the IBDV in this study is not comparable to chicken meat.

Kruk, Z et al. (2011) subjected chicken breast inoculated with *S*. typhimurium to HPP for 5 minutes at an initial temperature (of the pressure vessel) of 15 ±3 °C. The results of this study are in Table 1.

Table 1: Colony forming units (CFUs) per gram of *S*. typhimurium remaining after treatment at different pressures (MPa) for 5 minutes

Pressure (MPa)	CFU/g
0.1	6.17
300	5.53
450	2.82
600	Not detected (<2.0 log CFU/g)

Source: Kruk, Z et al. (2011)

The paper states that the 600 MPa treatment reduced the bacterial count (for the *E. coli*, *Listeria monocytogenes* and *S*. typhimurium strains tested) by between 6 and 8 $\log_{10}$ CFUs/g. However, as the detection limit appears to be 2 $\log_{10}$ CFU/g, a more accurate report of the log reduction achieved would have been a reduction of between 4.17 and 8 $\log_{10}$ CFU/g.

Kruk, ZA et al. (2014) investigated *S*. typhimurium inactivation in HPP-treated chicken breast meat (plain, and with added soy sauce and/or olive oil) using 300 MPa for 5 minutes at an initial temperature (of the pressure vessel) of 15 ±3 °C. The highest reduction of *S*. typhimurium was achieved in meat with added soy sauce (10 per cent weight for weight [w/w]), where *S*. typhimurium decreased from 6.17 CFU/g (control) to 5.5 CFU/g after treatment (a reduction of 0.67 CFU/g). In plain breast meat, a 0.64 CFU/g reduction was achieved, and in meat with both added soy sauce (5 per cent, w/w) and olive oil (5 per cent, w/w), *S*. typhimurium increased by 0.53 CFU/g after treatment. These findings again highlight how a product's composition can affect the efficacy of HPP treatment for inactivation of microorganisms.

Buckow et al. (2017) conducted preliminary studies on eight viruses—one strain each of AIV and NDV, and six strains of IBDV—providing evidence for how these virus strains react to HPP treatment in chicken meat homogenate. *Salmonella* strains were not tested in this study.

> Testing for IBDV is involved and labour intensive. The resource requirements for this type of research are a limitation to the scope of studies needed to fully understand the mechanisms and kinetics of virus inactivation … the underlying mechanism of pressure-induced virus inactivation is not fully understood yet. (Buckow et al. 2017)

2.3.1 Conclusions

- The evidence suggests that the sensitivity to HPP of different virus species, even different strains within the same species and serotype, is variable.
- Scientific evidence for the inactivation of various strains of pathogens of biosecurity concern by HPP of chicken meat is limited.
- The available evidence suggests that HPP treatment is effective at inactivating a range of microorganisms to varying degrees, but it depends on several factors.
- As further research is conducted on HPP, the underlying mechanisms and kinetics of pathogen inactivation may be better understood, offering a means to predict how differences between species and serotypes may affect HPP efficacy.

2.4 HPP efficacy in inactivation of microorganisms: specific assessment of the Buckow study

Sections 2.4.2–2.4.5 address comments raised in stakeholder submissions and through departmental assessment specific to how Buckow et al. (2017) conducted their study. Section 2.4.1 is an overview of the study.

2.4.1 Overview of the Buckow study

The Buckow et al. (2017) paper was provided as evidence for the efficacy of HPP inactivation of AIV, NDV and IBDV in chicken meat. Preliminary studies were conducted on:

- AIV strain A/chicken/Victoria/1/1985 (H7N7)
- NDV Herts strain
- IBDV strains Tasik94, CS88, variant E, GLS-5, APHIS and 52/70.

HPP was applied to chicken breast meat–virus homogenate samples at 600 MPa at room temperature for up to 120 seconds.

The meat–virus homogenate was prepared using 9 parts meat and 1 part buffer/virus suspension. The IBDV virus suspensions were derived from bursae of Fabricius, removed from SPF chickens 48 hours post-infection with IBDV. These were ground in a sterile pestle and mortar, and suspended in sterile phosphate-buffered saline (PBS) at a 1:5 ratio to make a 20 per cent suspension. This was centrifuged to separate ground tissue and the supernatant was stored in aliquots. For AIV and NDV suspensions, allantoic fluid was aspirated from inoculated embryonated chicken eggs to mix with the breast meat.

The meat component of the homogenate preparation was chicken breast meat from nonvaccinated SPF chickens that was ground in an electric blender. Aliquots of 9 g of ground meat were mixed with 1 ml of virus suspension and further ground with a pestle and mortar until the fluid and meat were fully homogenised. Batches of virus-homogenate were each split into five plastic sachets and sealed before treatment. From each batch one sub-sample was left untreated, for determination of start titres. Untreated samples were prepared, handled and tested as per the treated samples.

After treatment the samples were mixed with 10 ml of sterile PBS and homogenised using a sterile pestle and mortar. This mixture was centrifuged to pellet the muscle tissue and the supernatant extract was harvested for titration, to determine the reduction in titre of the viruses

in chicken meat homogenate after treatment. For IBDV variant E and GLS-5 strains, this was performed by inoculating serial dilutions into each of five chickens, which were euthanised two to three days later and their bursae harvested for histopathology and immunohistochemistry. Other IBDV strains were titrated in embryonated chicken eggs by chorioallantoic membrane (CAM) inoculation and antigen-capture ELISA (AC-ELISA) on CAM homogenates. Both AIV and NDV were titrated by inoculation into embryonated eggs by the allantoic sac route and testing of allantoic fluids by haemagglutination, with virus titres calculated by the Spearman-Karber method.

Table 2 shows the start and end titres of the viruses tested in Buckow et al. (2017). Both NDV and IBDV Tasik94 remained detectable in meat–virus homogenate samples after treatment.

Table 2 Reduction in titre of various avian viruses in chicken meat homogenate after treatment at 600 MPa and room temperature

Virus (strain)	Pressure holding time (s)	Titration method	Start titre (log_{10} EID_{50}/0.2 ml)	End titre (log_{10} EID_{50}/0.2 ml)
AIV (H7N7) Bendigo	60	AS	6.5	0
NDV (Herts)	120	AS	8.3	0.9
IBDV (Tasik94)	120	CAM	5.7	1.7
IBDV (CS88)	120	CAM	4.5	0
IBDV (APHIS)	120	CAM	4.3	0
IBDV (52/70)	120	CAM	3.2	0
IBDV (variant E)	15	Chick	2.1	0
IBDV (variant E)	120	Chick	≤2.7	0
IBDV (GLS-5)	15	Chick	2.5	0
IBDV (GLS-5)	120	Chick	≤2.8	0

AIV = avian influenza virus; EID = egg infectious dose; IBDV = infectious bursal disease virus; NDV = Newcastle disease virus

Notes:

AS—allantoic sac inoculation followed by haemagglutinating effect of allantoic fluid after embryo death or 5 days incubation.

CAM—chorioallantoic membrane inoculation followed by antigen-capture ELISA for IBDV on CAM after death of embryo or 7 days after inoculation.

Chick—inoculation onto the mucous membranes of 3-week-old chicks and histological analysis of bursa at 3 days after inoculation.

Source: Buckow et al. (2017)

Further experiments modelled the inactivation curve of IBDV Tasik94 strain. This showed a rapid initial drop of infectivity followed by a prolonged persistence and almost completely diminished inactivation rate (persistent 'tail' of resistant IBDV Tasik94). The effect of multiple pressure applications was also tested. It was determined that multiple-treated samples generally had higher or similar virus titres following treatment compared to samples that had been treated for a single cycle for similar total holding times.

Infectivity studies were also conducted for pressure-treated IBDV Tasik94 strain, as initial results indicated that viable virions remained in pressure-treated meat–virus homogenate. Undiluted meat extracts of four pressure-treated samples (600 MPa for 30 seconds) were each inoculated into five chickens by the mucosal route. The bursae were harvested two days after inoculation for testing by immunohistochemistry, ELISA and histopathology, to determine the presence or absence of infection. Additionally, meat extracts were titrated in eggs and assessed by embryo deaths and by AC-ELISA to confirm the amount of live virus remaining after pressure

treatment. Buckow et al. (2017) reported that inoculation did not infect chickens; however, subsequent titration in eggs found live virus in the samples. An untreated control sample was titrated in eggs to determine virus titre, however, the presence or absence of infection in chickens following inoculation with this control sample was not determined.

In summary, Buckow et al. (2017) assess how single strains of both AIV and NDV, and six strains of IBDV react to HPP treatment in chicken meat–virus homogenate samples. The study notes that the trials were preliminary and that findings can be useful for the application of HPP for limiting pathogen spread, specifically via chicken meat homogenate. However, there are several reasons why these findings cannot be extrapolated to commercial chicken meat products. These are discussed in the following sections.

2.4.2 Use of breast meat–virus homogenate

As previously outlined, the composition of a product will affect HPP treatment efficacy. Inactivation of pathogens is very likely to differ between commercial chicken meat products and meat–virus homogenate samples. Commercial chicken meat products vary in composition, but are not usually homogenised meat with added saline, and will potentially include skin, fat, bone and additives such as salt, sugar or marinades. The presence of any pathogens may be a result of infection (where pathogens may be free or contained within cells) or cross-contamination during processing.

Buckow et al. (2017) did not test any other carcase parts included in the chicken meat IRA definition of chicken meat. They state that their 'results cannot be extrapolated to other chicken tissues and non-meat products (for example, bone), as the inactivation kinetics may be very different in those tissues' (Buckow et al. 2017). Bone may be a significant reservoir of pathogens. Elankumaran, Heckert and Moura (2002) demonstrated that IBDV was readily isolated from homogenates of chicken bone marrow, bursa and caecal tonsils at two and four weeks post-infection at approximately equal titres. Abdul et al. (2013) showed that chicken bone marrow samples were positive for IBDV for up to 49 days post-infection.

Inactivation of pathogens may also differ between naturally and experimentally infected chicken meat. Thomas, King and Swayne (2008) studied the thermal inactivation of AIV and NDV in chicken meat and concluded that, under the conditions of their assay, highly pathogenic AIV was inactivated slower in meat from naturally infected chickens than in artificially infected chicken meat with a similar virus titre. However, highly pathogenic NDV was inactivated similarly in naturally and artificially infected meat. They hypothesised that the difference between AIV and NDV inactivation in artificially infected meat versus meat from infected chickens could be due to differences in viral replication in the host. There are currently no published studies comparing the efficacy of HPP inactivation of pathogens of biosecurity concern in naturally versus artificially infected chicken meat. Using only thermal processing as an indication, it is possible that the inactivation kinetics of HPP may differ for at least some pathogens of concern between naturally and artificially infected meats.

Conclusions

- It is unknown if the efficacy of HPP treatment differs between naturally and artificially infected tissues to inactivate pathogens of biosecurity concern.

- A pathogen replicating within an animal's cell after natural infection (even if experimental) may react differently to HPP treatment than a pathogen that has been added to extracellular fluid in an experimental setting—for example, using meat–virus homogenate.
- Buckow et al. (2017) state that their results cannot be extrapolated to other chicken tissues and non-meat products, as the inactivation kinetics may be very different in those tissues.
- Commercial chicken meat products are unlikely to comprise of homogenised breast meat, and will potentially include other muscle meat, skin, fat, bone and additives such as salt, sugar or marinades.
- These factors make it difficult to extrapolate the results of Buckow et al. (2017) to commercial chicken meat products.

2.4.3 Virus propagation and titration (quantitative determination of viruses) in chickens and eggs

Infectious bursal disease virus

As described in Section 2.4.1, to propagate IBDV virus, Buckow et al. (2017) euthanised chickens 48 hours post-inoculation, and to titrate variant E and GLS-5 strains, two to three days post-inoculation. The start titres reported in meat–virus homogenate for the six strains ranged from 2.1 $\log_{10}$ EID_{50}/0.2 ml (or even lower, as two values were reported as ≤2.7 and ≤2.8) to 5.7 $\log_{10}$ EID_{50}/0.2 ml (see Table 2).

Similar IBDV reference material reviewed by the department euthanised chickens at least 72 hours post-inoculation for virus isolation or to create virus stocks. Vindevogel, Meulemans and Halen (1975) found that IBDV persists for 10 days in the bursa of Fabricius and the highest viral concentrations are observed between day 4 and day 8 post-inoculation. Other example reference materials include:

- The World Organisation for Animal Health (OIE) recommends at least 72 hours for propagation of IBDV strains in chickens (OIE 2016). Although this is to prepare a positive-control antigen—not to produce working virus stocks—the chapter does stipulate that chickens should be euthanised 72–80 hours post-inoculation for virus isolation.
- The aquatic and terrestrial Australian and New Zealand standard diagnostic procedure for very virulent IBDV recommends isolation by inoculation of undiluted bursal homogenate to SPF chickens and recovery of virus from bursal tissue 3 days after inoculation (Ignjatovic 2004).
- Elankumaran, Heckert and Moura (2002) inoculated chickens with variant serotype 1 IBDV E/Delaware strain, orally and cloacally, and harvested their bursae three days post-inoculation to create a virus stock. The resulting stock used to infect chickens in the study had 5 $\log_{10}$ median TCID50/ml.
- Alexander and Chettle (1998) assessed heat inactivation of a field strain of IBDV. They euthanised chickens 72 hours post-inoculation with IBDV 52/70 strain for bursal harvest to create the virus stocks for heat treatment.
- Soubies et al. (2018) investigated the propagation and titration of IBDV, and harvested the bursae from chickens 4 days post-infection to create viral stocks.

Buckow et al. (2017) referenced the infectious bursal disease (IBD) chapter in the OIE Manual (OIE 2008) when describing the method for titrating IBDV variant E and GLS-5 strains in chickens. However, OIE recommendations regarding time between inoculation and euthanasia for bursal harvest for diagnostic purposes differ to the titration method used by Buckow et al.

(2017). The OIE Manual IBD chapter describes isolation of virus in chickens, whereby chickens are inoculated with samples prepared from bursae of infected birds. The chickens are killed 72–80 hours after inoculation, and their bursae of Fabricius examined. The chapter states that the extent of bursal damage may vary considerably with the pathogenicity of the studied IBDV strain. Buckow et al. (2017) euthanised chickens 2–3 days post-inoculation for bursal harvest.

Furthermore, the start titres of variant E and GLS-5 strains in untreated chicken meat homogenate were reported as ≤2.7 $\log_{10}$ egg infectious dose $(EID)_{50}$ and ≤2.8 $\log_{10}$ EID_{50}, respectively (see Table 2). Due to the reporting method chosen—'less than or equal to' values, rather than stating the (mean titre) ± (inclusive range of titre values)—it may be possible that the start titre determined (for the untreated samples) for 1–4 of the inoculated chicks for each strain was very low or beyond the limit of detection.

It is also unclear exactly how start and end titres were determined for variant E and GLS-5 meat–virus homogenates. Bursae were harvested for histopathology (sections of bursa were stained with haematoxylin and eosin to detect the characteristic lesions of IBDV) and immunohistochemistry (for detection of IBDV antigen, viewed under standard light microscopy). However, results were reported in EID_{50}.

For IBDV strains Tasik94, CS88, APHIS and 52/70, Buckow et al. (2017) describe the titration method by CAM inoculation of 9–11-day-old embryonated chicken eggs, again referencing the OIE Manual IBD chapter. The OIE Manual IBD chapter recommends using five 9–11-day-old embryos for CAM inoculation (in addition to five 6–8-day-old embryos for yolk sac inoculation). Buckow et al. (2017) inoculated 3–5 embryonated eggs for each dilution using CAM inoculation only.

The use of the AC-ELISA to quantify IBDV strains Tasik94, CS88, APHIS and 52/70 also gives rise to other considerations. A study compared AC-ELISA with conventional methods for titration of IBDV and found that 'the use of AC-ELISA for diagnostic or epidemiological purposes would be limited by its lower sensitivity, which necessitates that other viral detection systems should be considered for diagnosis in addition to AC-ELISA' (Hassan, Saif & Shawky 1996). They also found that a polyclonal system is more sensitive than a monoclonal system when both are used to titrate the same sample, and Buckow et al. (2017) used a monoclonal system. The AC-ELISA was run in duplicate; however, they did not report the coefficient of variation (the ratio of the standard deviation to the mean). This may have helped the reader to assess the reliability of the assay (level of variance between duplicate samples).

Molecular assays based on reverse-transcription polymerase chain reaction (RT-PCR) technology to increase the sensitivity of virus detection may have been useful, particularly when starting titres were low. Jackwood (2004) found that RT-PCR assays are more versatile, sensitive and specific than AC-ELISA. *Fenner's Veterinary Virology* (MacLachlan & Dubovi 2016) states that detecting the IBDV genome using RT-PCR is now routine. Real-time PCR was found to be at least 10 times more sensitive than ELISA for detecting IBDV (Aini et al. 2008). If PCR was used, it would need to be a method that differentiated nucleic acids associated with viable cells from those associated with cells inactivated by the HPP treatment. Viability PCRs, which can distinguish between live and dead cells via a method involving pre-treatment of samples, are available for various applications. However, it is unknown if this type of analytical approach could have been suitably applied in Buckow et al. (2017).

Avian influenza virus and Newcastle disease virus

Buckow et al. (2017) describe the method for titration of AIV and NDV in eggs, referencing the AI chapter in the OIE Manual (OIE 2008). The OIE Manual states that 'fluids that give a negative reaction should be passaged into at least one further batch of eggs' (OIE 2008). In Buckow et al. (2017), an unspecified number of 9–11-day-old embryonated eggs were inoculated via the allantoic sac route. Allantoic fluids from all eggs found dead after day one, and all eggs that survived five days, were tested by haemagglutination. A second passage for eggs with negative reactions was not mentioned.

It has been shown that a second egg passage can increase the haemagglutination titre of AI and help with other diagnostic work (Dormitorio et al. 2009). It does not appear that Buckow et al. (2017) performed a second passage for negative fluids and, without this step, the end titre values reported for AIV and NDV could be misrepresentative.

All viruses tested

For virus titration, readers would have known the sensitivity of the diagnostic methods employed if the limits of detection were provided. For the ELISA, 'the cut-off was calculated as double the OD [optical density] values of the negative CAM control values, as determined by previous validation of this assay (unpublished data)' (Buckow et al. 2017). Buckow et al. (2017) did not discuss the limit of detection for virus quantification by bursal histopathology and immunohistochemistry.

Conclusions

- The IBDV start titres in the meat–virus homogenate (Buckow et al. 2017) may have been higher had the chickens used for bursal harvest been allowed to live for at least 72 hours post-inoculation, rather than 48 hours. Low start titres offer less evidence of the inactivation capability of HPP in these instances.
- For IBDV titration in chickens, ideally all birds should have been euthanised at the same time point—not in the range of 2–3 days post-inoculation in Buckow et al. (2017)—to ensure that titres were comparable.
- Euthanasia at 2–3 days post-inoculation may have affected the end titres determined by these studies and provided negative results. Allowing the chickens to live for at least 72 hours post-inoculation as per OIE recommendations and methods established in other published research would have offered more appropriate time for IBDV replication and histological bursal changes to occur.
- Titration in eggs by OIE-recommended methods, where applicable, would have also helped to determine end titre values.
- The above factors may have resulted in an inaccurate representation of actual log reduction of virus strains achieved by HPP treatment.
- The use of molecular testing, provided suitable viability PCR technology could have been applied, may have increased confidence regarding the presence or absence of viable virus in treated chicken meat homogenate samples.

2.4.4 Infectivity studies

Infectious bursal disease virus

Infectivity studies using IBDV Tasik94 strain found no evidence of infection in inoculated chickens, despite finding a mean titre of 1.4 $\log_{10}$ EID_{50}/0.2 ml (range 1.2–1.5 $\log_{10}$ EID_{50}/0.2 ml) in inoculated eggs. Thus, the infectivity study results are preliminary, and:

> Further research on the infectivity for chickens following high pressure treatment would be valuable … Any further testing would benefit from inoculating an untreated control sample. In addition, it would be beneficial to rule out the possibility that infection of chickens with treated virus may be present in an atypical manner. Extending the trial beyond two days to allow further virus replication and host seroconversion, and also passage of bursal material to other susceptible chickens, would help support this. (Buckow et al. 2017)

The OIE Manual process for IBDV isolation of virus in chickens has been outlined previously, and recommends euthanasia 72–80 hours after inoculation for bursal examination (OIE 2008). Another paper outlined the detection of viral antigens of IBDV in the bursa of Fabricius, stating:

> The viral antigens specific to IBDV may be detected by direct and indirect immunofluorescence or by immunoperoxidase staining in the bursal follicles of infected chickens between the fourth and sixth day after inoculation. No viral antigen is detectable from the tenth day. However, the virus can be isolated from bursae sampled from the second to the tenth day, with a maximum infectious titre after four days. (Van Den Berg 2000)

Buckow et al. (2017) euthanised chickens two days post-inoculation and tested for IBDV using histology of the bursa (to detect IBDV lesions), and immunohistochemistry and ELISA (to detect IBDV antigens). As antigens may not reach their maximum titre until the fourth day post-inoculation, the shorter time frame between inoculation and bursal harvest in this study may have affected the results. Other studies have found similar doses of IBDV capable of causing infection in chickens; however, the time between inoculation and euthanasia was longer:

- Ismail and Saif (1991) showed that a dose of 2 $\log_{10}$ EID_{50} of IBDV variant E strain, and a dose of 2 $\log_{10}$ EID_{50} of IBDV STC strain both resulted in 100 per cent infection of chickens. In these experiments, chickens were euthanised at 5 and 10 days post-inoculation for testing.
- Eldaghayes et al. (2006) showed that a dose of 1.7 $\log_{10}$ EID_{50} of IBDV 52/70 strain and a dose of 1.3 $\log_{10}$ EID_{50} of IBDV UK661 strain resulted in infection of inoculated chickens. In this study, bursal tissue was removed at 24 hours post-challenge and at 12-hour intervals for three days after, so the viral load could be quantified by RT-PCR. After infection, viral load increased, reaching a maximum at 72 hours post-infection (strain 52/70) and 84 hours post-infection (strain UK661).

Furthermore, clinical progression of IBDV tends to be faster at higher infecting doses and slower at lower infecting doses (noting that virulence differs greatly between strains, which also affects clinical progression). Jackwood et al. (2009) found that inoculation of chickens with two different doses of two very virulent IBDV stains (rA and rB) resulted in different disease progression. For both strains, a dose of 5.5 $\log_{10}$ EID_{50} resulted in all chickens being moribund at 48 hours post-infection, whereas at a lower dose of 2 $\log_{10}$ EID_{50}, most chickens appeared healthy at 48 hours. Jackwood and Sommer-Wagner (2010) found that the dose did not affect the severity of the disease, but lower doses did delay the onset of lesions.

Buckow et al. (2017) hypothesised that the inability for residual Tasik94 virions to cause infection in eggs, but not chickens, may be due to subtle changes to the viral proteins under high pressure that allow them to cause infection in the mild environment of a chicken embryo, but not in the immunologically hostile environment of mucous membranes. The study stated that:

> Although [they] found no disease in a trial of four treated samples inoculated into 20 birds, these results should be regarded as preliminary. Further research on the infectivity for chickens following high pressure treatment would be valuable.
>
> … the titres of the inocula were similar in both (8.8 and 8.0 $\log_{10}$ ID_{50}/0.2 ml in eggs and chickens, respectively) showing that there was no observed difference when comparing the sensitivity of chickens and eggs. Therefore, the absence of infection in chickens after inoculation with treated IBDV-Tasik94 is not due to lower sensitivity of chickens for this virus. (Buckow et al. 2017)

However, the difference in titre (0.8 $\log_{10}$ ID_{50}/0.2 ml) equates to a difference of approximately 531 million viral copies—6.3 times more viral copies in the inocula used for eggs.

Newcastle disease virus

NDV Hertz strain was also present at a low titre in meat–virus homogenate after HPP treatment (600 MPa for 120 seconds), but infectivity was not studied. It is reasonable to assume that the statements in Buckow et al. (2017) regarding the value of further infectivity research, inoculation of an untreated control sample, atypical infection and trial extension for IBDV would also apply to NDV Hertz.

Conclusions

- An infectious dose of 1.2–1.5 $\log_{10}$ EID_{50} Tasik94 strain may not be enough to result in bursal lesions or a detectable level of IBDV antigen in bursal tissue by ELISA or immunohistochemistry at two days post-inoculation; however, the birds may have been infected.
- To better determine the infectivity of residual IBDV after HPP processing in Buckow et al. (2017), inoculated chickens should be allowed to live for a minimum of 72 hours post-inoculation before culling, harvesting and testing bursal tissue.
- Ideally, a subset of inoculated chickens would have been allowed to live longer, and culling, bursal harvesting and testing done at intervals between 84 and 144 hours. This would account for varying levels of antigen in bursal tissue and potential atypical infection.
- Using a control is best practice for all viruses undergoing infectivity studies to confirm that the original virus stock is infective. Chickens should have been inoculated with the untreated control sample used in infectivity studies in Buckow et al. (2017), to ensure that the homogenate was infectious to chickens before treatment.
- Molecular diagnostics would have been useful in the infectivity study to increase the sensitivity of detection.
- Infectivity studies for NDV would have been useful to assess the infectivity of residual NDV after treatment.
- A finding of viable virus via egg inoculation would be considered a positive result for biosecurity purposes—for example, in imported commodities such as live birds.

2.4.5 Other considerations

Inactivation modelling

Buckow et al. (2017) presented an inactivation curve of IBDV Tasik94 strain in meat–virus homogenate, and reported approximately 27 data points from samples of meat–virus homogenate undergoing HPP at 600 MPa for 0–150 seconds. It appears that the authors tested:

- 20 samples at 0–30 seconds of treatment
- 3 samples at 30–60 seconds of treatment
- 1 sample at 90 seconds of treatment
- 1 sample at 120 seconds of treatment
- 2 samples at 150 seconds of treatment.

Relatively few results (n = 4) are provided for treatment times of 90 seconds or more, but the tailing effect (persistence of virus) is clear. Buckow et al. (2017) report a 5–6 $\log_{10}$ EID_{50}/0.2 ml reduction within 10–15 seconds. Two of the four results reported between 90 and 150 seconds fail to achieve a 6 $\log_{10}$ reduction. Of interest was the variation in log reduction—for example, the log reduction reported for one sample after 150 seconds of HPP treatment was 1.5 $\log_{10}$ EID_{50}/0.2ml less than that reported for two samples tested at 45 and 120 seconds, despite using a single batch of IBDV Tasik94 suspension to minimise treatment variation.

Buckow et al. (2017) used time 0 as the point at which 600 MPa was attained. There appears to be a cluster of data points at time 0, including one of >1 $\log_{10}$ EID_{50}/0.2 ml (data point partially shown on graph). It is possible that several samples tested at time 0 were used to form the mean start titre that the mean virus titre loss was calculated from. However, the data points reported were not explained, including the number tested at each time point, which would have helped readers to interpret the pressure inactivation results.

Virus titres used—worse-case scenarios

The study stated:

> Although some avian influenza virus subtypes may occur in large quantities in skeletal muscle, generally IBDV and NDV do not replicate in this tissue type. Large virus amounts will, therefore, only occur in meat through surface contamination, with bursal tissue in the case of IBDV, for example. The virus levels used in this study can only be achieved by mixing muscle tissue with other tissue that contains high titres, and, therefore, present a somewhat artificial scenario. The method of mixing virus with meat, as done in this study, although artificial, does approach the problem with a level of conservativism that allows applicability to worse-case scenarios. (Buckow et al. 2017)

Cross-contamination during processing is not the only potential source of similar titres of IBDV and NDV in chicken meat (as per the definition in the IRA). IBDV can be readily isolated from chicken bone marrow and other tissues from infected chickens (Abdul et al. 2013; Elankumaran, Heckert & Moura 2002; Vindevogel, Meulemans & Halen 1975). Specific to skeletal muscle, Abdul et al. (2013) detected IBDV in breast muscle of SPF chickens inoculated separately with 4 $\log_{10}$ EID_{50}/0.2 ml of IBDV strains STC and IN, at titres of 2.69 ±0.014 and 3.02 ±0.32, respectively, at 3 days post-infection, and for IN strain, 2.83 ±0.61 at 10 days post-infection (titres reported as average log of relative viral RNA copy number per volume tissue homogenate).

Alexander, Manvell and Parsons (2006) showed that the highest titres of NDV Herts 33/56 strain were recorded at day 4 post-inoculation, with 6 $\log_{10}$ median EID_{50}/g recorded in a pool of heart, kidney and spleen tissue; 4.2 $\log_{10}$ EID_{50}/g in the leg muscle; and 4 $\log_{10}$ EID_{50}/g in the breast muscle and faeces. Thomas, King and Swayne (2008) found titres of NDV CA/02 strain to

be 6.8 $\log_{10}$ EID_{50}/g in thigh meat and 6.4 $\log_{10}$ EID_{50}/g in breast meat, in chickens 3–5 days post-inoculation. These studies suggest that the inference that large amounts of virus will only occur in meat through surface contamination may not be correct.

Furthermore, the start titres determined for several IBDV strains are possibly lower than titres that occur in meat of naturally infected chickens. However, this possibility is based on few reports, as there are few published data where IBDV titres in infected chicken meat have been determined—particularly for the strains that Buckow et al. (2017) used with reported start titres of 3.2 $\log_{10}$ EID_{50}/0.2 ml or less (strains 52/70, variant E and GLS-5).

Conclusions

- The pressure inactivation kinetic study of IBDV Tasik94 strain shows a tailing effect (persistence of virus at all treatment times).
- Cross-contamination during processing is not the only potential source of virus in chicken meat. Large amounts of virus may be present within tissues.
- Additional studies could assist in determining whether the start titres for IBDV strains in Buckow et al. (2017) are higher, similar or lower than titres that occur in meat of naturally infected chickens. This would help compare the study results with real-life scenarios.

3 Conclusion and recommendations

The chicken meat IRA recommends risk management measures for nine pathogens: high pathogenicity AIV, low pathogenicity AIV, NDV, very virulent IBDV, exotic antigenic variant strains of IBDV, *S.* pullorum, *S*. gallinarum, *S*. enteritidis and multidrug-resistant strains of *S*. typhimurium.

The currently available scientific literature on HPP inactivation of these pathogens of biosecurity concern is limited. The department is also of the opinion that such literature is preliminary. Importantly, the underlying mechanism of pressure-induced virus inactivation has not yet been elucidated. Studies suggest that HPP treatment is effective at inactivating a range of microorganisms to varying degrees, but depends on several factors. However, at this stage, there is an insufficient body of evidence to allow the department to develop import conditions that manage the risk for all pathogens of concern to an appropriate level in the variety of chicken meat products that fall under the chicken meat IRA definition.

Consequently, HPP of chicken meat is not considered to be a suitable alternative risk management measure to the heat treatments in the chicken meat IRA.

3.1 Future applications

For the department to consider a particular HPP treatment as a suitable risk management measure for pathogens of concern in chicken meat products, the following would need to be submitted as an import proposal:

- details of the HPP treatment applied, including the
  - pressure range
  - hold time
  - temperature range
- details of the product composition, including the
  - cut of meat, skin on/off, bone-in/out
  - pH range
  - fat content
  - water activity
  - additives, including salt and sugar
  - size/weight range
- which pathogens of biosecurity concern the HPP treatment is intended as a risk management measure for
- peer-reviewed scientific literature showing that HPP treatment, under the specific parameters outlined in the proposal, is effective at inactivating pathogens of biosecurity concern (where risk management is sought) in products of the composition outlined in the proposal or in representative products. The findings of this report need to be considered when deciding what would constitute appropriate and sufficient scientific literature
- country of origin.

Regarding the last point (country of origin), the department has procedures in place for granting market access to countries for the purposes of export of chicken meat to Australia to ensure that Australia's requirements can be met. The country approval process is complex, and the exporting country's competent veterinary authority must support the process and help provide

required information and facilitate in-country verification activities. With this process in mind, import proposals should also include information on the proposed country of origin. Australia has trade relationships with many countries where processes are in place to manage market access request prioritisation across a range of animal and plant commodities. Due to the complexities of the country approval process for chicken meat imports, the proposal is more likely to be assessed as a higher priority if the prospective exporting country's competent veterinary authority shows interest, in the form of an import proposal or during bilateral trade discussions.

Appendix A Current certification requirements for imported chicken meat

The following is an excerpt from *Generic import risk analysis for chicken meat: final import risk analysis report* (Biosecurity Australia 2008).

1.1 Each consignment must be accompanied by a Government Veterinary Certificate in accordance with the Office International des Epizooties (OIE) International Terrestrial Animal Health Code 'Model international veterinary certificate for meat of domestic animals' signed by an Official Government Veterinarian. The certificate must provide details of:

- the packaging of the meat including details of the labelling,
- the addresses and veterinary approval numbers of establishments at which the animals from which the meat was derived were slaughtered, the cutting-up establishment at which it was prepared and the establishment at which it was stored prior to export,
- the names and addresses of the exporter and the consignee.

1.2 The Official Government Veterinarian of the source country must certify in English, under

IV. Attestation of wholesomeness, that:

1.2.1 The chickens from which the meat was derived have been continuously resident in the source country since hatching and were slaughtered on ..... (dates).

1.2.2 The chickens from which the meat was derived passed ante- and post-mortem veterinary inspection under official veterinary supervision, and the meat is considered to be fit for human consumption.

1.3. All of the following risk management measures apply:

a. Highly pathogenic avian influenza virus

EITHER

(i) The chickens from which the meat was derived have been kept since hatching in a country or zone which is recognised by Australian Government authorities as free of highly pathogenic notifiable avian influenza (HPNAI).

OR

(ii) The chicken meat has been processed to ensure the destruction of the AI virus and has been heated to a minimum core temperature of 70 °C for at least one minute (or time/temperature equivalent).

b. Low pathogenicity notifiable avian influenza virus

EITHER

(i) The chickens from which the meat was derived have been kept since hatching in a country or zone which is recognised by Australian Government authorities as free of low pathogenicity notifiable avian influenza (LPNAI).

OR

(ii) The chicken meat has been processed to ensure the destruction of the AI virus and has been heated to a minimum core temperature of 70 °C for at least one minute (or time/temperature equivalent).

OR

(iii) Neither paragraph b.(i) nor b.(ii) is applicable to this consignment.

Note: In this case, the meat must be processed in Australia to ensure destruction of AI virus.

c. Newcastle disease virus

EITHER

(i) The chickens from which the meat was derived have been kept since hatching in a country or zone which is recognised by Australian Government authorities as free from Newcastle disease.

AND

Any live vaccines used on chickens from which the meat was derived were produced from lentogenic strains of Newcastle disease virus.

OR

(ii) The chicken meat has been processed to ensure the destruction of the Newcastle disease virus and has been heated to a minimum core temperature of 70 °C for at least 8.2 minutes (or time/temperature equivalent).

OR

(iii) Neither paragraph c.(i) nor c.(ii) is applicable to this consignment.

Note: In this case, the meat must be processed in Australia to ensure destruction of ND virus.

d. Very virulent infectious bursal disease virus (vvIBD)

EITHER

(i) The chickens from which the meat was derived have been kept since hatching in a country or zone which is recognised by Australian Government authorities as free from very virulent infectious bursal disease (vvIBD)

OR

(ii) The chicken meat has been processed to ensure the destruction of the vvIBD virus to the satisfaction of Australian Government authorities. The product must be heated to a minimum core temperature of 80 °C for at least 125 minutes (or time/temperature equivalent).

OR

(iii) Neither paragraph d.(i) nor d.(ii) is applicable to this consignment.

Note: In this case, the meat must be processed in Australia to ensure destruction of vvIBD virus.

e. Exotic antigenic variant strains of IBD virus

For the purposes of this document, exotic antigenic variant strains are defined as variant strains that are antigenically and genetically different from those that exist in Australia, and include United States variant strains.

EITHER

(i) The chickens from which the meat was derived have been kept since hatching in a country or zone which is recognised by Australian Government authorities as free from exotic antigenic variant infectious bursal disease (var IBD).

OR

(ii) The chicken meat has been processed to ensure the destruction of the var IBD virus to the satisfaction of Australian Government authorities. The product must be heated to a minimum core temperature of 80 °C for at least 125 minutes (or time/temperature equivalent).

OR

(iii) Neither paragraph e.(i) nor e.(ii) is applicable to this consignment.

Note: In this case, the meat must be processed in Australia to ensure destruction of var IBD virus.

f. Salmonella Gallinarum (fowl typhoid) & Salmonella Pullorum (pullorum disease)

EITHER

(i) The chickens from which the meat was derived have been kept since hatching in a country or zone which is recognised by Australian Government authorities as free from fowl typhoid and pullorum disease.

OR

(ii) The chicken meat has been processed to ensure the destruction of S. Pullorum and S. Gallinarum and has been held at a minimum core temperature of 70 °C for at least 2.5 minutes (or time/temperature equivalent).

OR

(iii) Neither paragraph f.(i) nor f.(ii) is applicable to this consignment.

Note: In this case, the meat must be processed in Australia to ensure destruction of Salmonella organisms.

g. Salmonella Enteritidis and multi-drug resistant S. Typhimurium

EITHER

(i) The chickens from which the meat was derived have been kept since hatching in a country or zone which is recognised by Australian Government authorities as free from S. Enteritidis and multi-drug resistant S. Typhimurium.

OR

(ii) The chicken meat has been processed to ensure the destruction of S. Enteritidis and multidrug resistant S. Typhimurium and has been held at a minimum core temperature of 70 °C for at least 2.5 minutes (or time/temperature equivalent).

OR

(iii) Neither paragraph g.(i), nor g.(ii) is applicable to this consignment.

Note: In this case, the meat must be processed in Australia to ensure destruction of Salmonella organisms.

1.4 The establishment where the chickens from which the meat was derived were slaughtered, the establishment where the meat was prepared and the establishment where it was stored, have current AQIS approval for facilities and hygienic operation;

Note: The name(s), address(es) and veterinary control number(s) of plant(s) must be specified;

1.5 Officials of the Veterinary Authority of the source country were present in plants at all times when chickens were being slaughtered for export to Australia.

1.6 A quality assurance program is in place to ensure that poultry/meat destined for export to Australia is kept separate from poultry/meat not destined for Australia, and is handled in such a way as to ensure that there is no cross-contamination.

1.7 Product destined for Australia was processed and produced following a complete clean and sanitisation of the entire processing plant, and before product not destined for export to Australia.

1.8 The meat has been prepared for export and packed on ..... (dates) in bags, wrappers or packing containers which were clean and new, and in a manner which prevented contamination.

1.9 The identification number of the slaughtering establishment and/or the establishment where the meat was prepared is readily visible on the package or wrapping containing the meat, in such a way that the numbers cannot readily be removed without damaging the package or wrapping.

1.10 The meat was not exposed to contamination prior to export.

1.11 The meat is being transported to Australia in a clean container sealed with an Official Government seal; the container contains only meat eligible for entry into Australia.

References

The following references are divided into those provided via the stakeholder consultation process, and additional references sought by the department during its assessment.

Provided via stakeholder submission:

AHA 2009, *AUSVETPLAN Disease Strategy: Infectious bursal disease caused by very virulent IBD virus or exotic antigenic variant strains of IBD virus*. Version 3.0, Animal Health Australia, Canberra.

Alexander, DJ & Chettle, NJ 1998, 'Heat inactivation of serotype 1 infectious bursal disease virus', *Avian Pathology*, vol. 27, no. 1, pp. 97–9.

Alexander, DJ, Manvell, RJ & Parsons, G 2006, 'Newcastle disease virus (strain Herts 33/56) in tissues and organs of chickens infected experimentally', *Avian Pathology*, vol. 35, no. 2, pp. 99–101.

Ali, S, Kang, G & Joo, S 2008, 'A review: influences of pre-slaughter stress on poultry meat quality', *Asian–Australasian Journal of Animal Sciences*, vol. 21, pp. 912–6.

Araud, E, DiCaprio, E, Yang, Z, Li, X, Lou, F, Hughes, J, Chen, H & Li, J 2015, 'High-pressure inactivation of rotaviruses: role of treatment temperature and strain diversity in virus inactivation', *Applied and Environmental Microbiology*, vol. 81, no. 19, pp. 6669–78.

Australian Government 2009, Fact sheet: Biosecurity policy determination for chicken meat, Australian Government, viewed 4 February 2019.

Bedale, W 2014, *Use of high pressure processing (HPP) with antimicrobials in food*, Food Research Institute, University of Wisconsin-Madison, Madison.

Berri, C, Debut, M, Sante-Lhoutellier, V, Arnould, C, Boutten, B, Sellier, N, Baeza, E, Jehl, N, Jego, Y, Duclos, MJ & Le Bihan-Duval, E 2005, 'Variations in chicken breast meat quality: implications of struggle and muscle glycogen content at death', *British Poultry Science*, vol. 46, no. 5, pp. 572–9.

Biosecurity Australia 2008, *Generic import risk analysis report for chicken meat: final report*, Australian Government, Canberra.

Buckow, R, Bingham, J, Daglas, S, Lowther, S, Amos-Richie, R & Middleton, D 2017, 'High pressure inactivation of selected avian viral pathogens in chicken meat homogenate', *Food Control*, vol. 73, Part B, pp. 215-22.

Buckow, R, Isbarn, S, Knorr, D, Heinz, V & Lehmacher, A 2008, 'Predictive model for inactivation of feline calicivirus, a norovirus surrogate, by heat and high hydrostatic pressure', *Applied and Environmental Microbiology*, vol. 74, no. 4, pp. 1030–8.

Burleson, F, Chambers, T & Wiedbrauk, D 1992, *Virology. A laboratory manual*, Academic Press. Harcourt Brace Jovanovich Publishers, San Diego.

Considine, KM, Kelly, AL, Fitzgerald, GF, Hill, C & Sleator, RD 2008, 'High-pressure processing—effects on microbial food safety and food quality', *FEMS Microbiology Letters*, vol. 281, no. 1, pp. 1–9.

D'Souza, T, Karwe, M & Schaffner, DW 2014, 'Effect of high hydrostatic pressure on *Salmonella* inoculated into creamy peanut butter with modified composition', *Journal of Food Protection*, vol. 77, no. 10, pp. 1664–8.

Daryaei, H, Yousef, A & Balasubramaniam, V 2016, 'Microbial aspect of high pressure processing', in V Balasubramaniam, G Barbosa-Cánovas & H Lelieveld (eds), *High pressure processing of foods. Principals, technology and applications*, Springer, New York, NY.

Durairaj, V, Linnemann, E, Icard, AH, Williams, SM, Sellers, HS & Mundt, E 2013, 'An in vivo experimental model to determine antigenic variations among infectious bursal disease viruses', *Avian Pathology*, vol. 42, no. 4, pp. 309–15.

Elankumaran, S, Heckert, RA & Moura, L 2002, 'Pathogenesis and tissue distribution of a variant strain of infectious bursal disease virus in commercial broiler chickens', *Avian Diseases*, vol. 46, no. 1, pp. 169–76.

Eldaghayes, I, Rothwell, L, Williams, A, Withers, D, Balu, S, Davison, F & Kaiser, P 2006, 'Infectious bursal disease virus: strains that differ in virulence differentially modulate the innate immune response to infection in the chicken bursa', *Viral Immunology*, vol. 19, no. 1, pp. 83–91.

Eteradossi N & Saif M 2008, 'Infectious bursal disease', in Saif YM (ed.), *Diseases of poultry*, 12th ed, Iowa State University Press, Iowa City.

FAO & WHO 1993, *International food standards (Codex Alimentarius)*, Food and Agriculture Organization, Rome.

Farkas DF & Hoover DG 2000, 'High pressure processing', *Journal of Food Science*, vol. 65, no. s8, pp. 4–108.

FSIS 2012, *Directive 6120.2: High pressure processing (HPP) and inspection program personnel (IPP) verification responsibilities*, US Food Safety and Inspection Service, Washington DC.

— 2014, *Food Safety Compliance Guideline: Controlling* Listeria monocytogenes *in post-lethality exposed ready-to-eat meat and poultry products*, US Food Safety and Inspection Service, Washington DC.

Giddings NJ, Allard HA & Hite BH 1929, 'Inactivation of the tobacco mosaic virus by high pressure', *Phytopathology*, vol. 19, pp. 749–50.

Heine, HG, Haritou, M, Failla, P, Fahey, K & Azad, A 1991, 'Sequence analysis and expression of the host-protective immunogen VP2 of a variant strain of infectious bursal disease virus which can circumvent vaccination with standard type I strains', *Journal of General Virology*, vol. 72 (Pt 8), pp. 1835–43.

Hiperbaric 2014, Hiperbaric white paper: HPP on raw chicken meat, Hiperbaric, Florida.

Hirneisen, K, Black, E, Cascarino, J, Fino, V, Hoover, D & Kniel, K 2010, 'Viral inactivation in foods: a review of traditional and novel food-processing technologies', *Comprehensive Reviews in Food Science and Food Safety*, vol. 9, pp. 3–20.

Huang HW, Wu SJ, Lu JK, Shyu YT & Wang CY 2017, 'Current status and future trends of high-pressure processing in food industry', *Food Control*, vol. 72, pp. 1–8.

Hwang CA & Fan X 2015, 'Processing quality and safety of irradiated and high pressure-processed meat and seafood products', in Siddiqui MW & Rahman MS (eds), *Minimally processed foods: Technologies for safety, quality, and convenience*, Springer, Switzerland.

Ignjatovic J & Prowse S 1997, *Infectious bursal disease virus (IBDV): To determine if current vaccination strategies prevent the emergence of variant IBDV strains in Australia*. A report for the Rural Industries Research and Development Corporation. Project No. CSK-3AJ, Rural Industries Research and Development Corporation, Wagga Wagga.

Isbarn, S, Buckow, R, Himmelreich, A, Lehmacher, A & Heinz, V 2007, 'Inactivation of avian influenza virus by heat and high hydrostatic pressure', *Journal of Food Protection*, vol. 70, no. 3, pp. 667–73.

Ismail, NM & Saif, YM 1991, 'Immunogenicity of infectious bursal disease viruses in chickens', *Avian Diseases*, vol. 35, no. 3, pp. 460–9.

Jackwood, DJ, Sommer-Wagner, SE, Stoute, AS, Woolcock, PR, Crossley, BM, Hietala, SK & Charlton, BR 2009, 'Characteristics of a very virulent infectious bursal disease virus from California', *Avian Diseases*, vol. 53, no. 4, pp. 592–600.

Jackwood, DJ, Stoute, ST & Crossley, BM 2016, 'Pathogenicity of genome reassortant infectious bursal disease viruses in chickens and turkeys', *Avian Diseases*, vol. 60, no. 4, pp. 765–72.

Khadre, MA & Yousef, AE 2002, 'Susceptibility of human rotavirus to ozone, high pressure, and pulsed electric field', *Journal of Food Protection*, vol. 65, no. 9, pp. 1441–6.

Kingsley, DH 2013, 'High pressure processing and its application to the challenge of virus-contaminated foods', *Food and Environmental Virology*, vol. 5, no. 1, pp. 1–12.

Kingsley, DH, Chen, H & Hoover, DG 2004, 'Inactivation of selected picornaviruses by high hydrostatic pressure', *Virus Research*, vol. 102, no. 2, pp. 221–4.

Kingsley, DH, Holliman, DR, Calci, KR, Chen, H & Flick, GJ 2007, 'Inactivation of a norovirus by high-pressure processing', *Applied and Environmental Microbiology*, vol. 73, no. 2, pp. 581–5.

Kingsley, DH, Meade, GK & Richards, GP 2002, 'Detection of both hepatitis A virus and Norwalk-like virus in imported clams associated with food-borne illness', *Applied and Environmental Microbiology*, vol. 68, no. 8, pp. 3914–8.

Kruk, Z, Yun, H, Rutley, D, Lee, E, Kim, Y & Jo, C 2011, 'The effect of high pressure on microbial population, meat quality and sensory characteristics of chicken breast fillet', *Food Control*, vol. 22, pp. 6–12.

Kumar, R & Rani, M 2014, 'Chemical composition of chicken of various commercial brands of chicken in market', *IOSR Journal of Agricultural and Veterinary Science*, vol. 7, pp. 22–6.

Lee A & Grove S 2016, 'Virus inactivation during food processing', in Goyal SM & Cannon JL (eds), *Viruses in foods*, 2nd edition, Springer, Switzerland.

Lien R, Bilgili S, Hess J & Joiner K 2012, 'Induction of deep pectoral myopathy in broiler chickens via encouraged wing flapping', *Journal of Applied Poultry Research*, vol. 21, pp. 556–62.

Lou, F, Neetoo, H, Chen, H & Li, J 2015, 'High hydrostatic pressure processing: a promising nonthermal technology to inactivate viruses in high-risk foods', *Annual Review of Food Science and Technology*, vol. 6, pp. 389–409.

Lou, F, Neetoo, H, Li, J, Chen, H & Li, J 2011, 'Lack of correlation between virus barosensitivity and the presence of a viral envelope during inactivation of human rotavirus, vesicular stomatitis virus, and avian metapneumovirus by high-pressure processing', *Applied and Environmental Microbiology*, vol. 77, no. 24, pp. 8538–47.

MacLachlan, N & Dubovi, Ee 2016, *Fenner's veterinary virology*, fifth edition, Elsevier.

Morales, P, Calzada, J, Rodriguez, B, De Paz, M & Nunez, M 2009, 'Inactivation of *Salmonella* enteritidis in chicken breast fillets by single-cycle and multiple-cycle high pressure treatments', *Foodborne Pathogens and Disease*, vol. 6, no. 5, pp. 577–81.

NSW Food Authority 2016, *High pressure processing: Product considerations in HPP*, NSW Department of Primary Industries Food Authority, Sydney.

OIE 2000, 'Infectious bursal disease (Gumboro disease)', *Revue scientifique et technique (International Office of Epizootics)*, vol. 19, no. 2, pp. 527–43.

— 2015, 'Avian influenza (infection with avian influenza viruses)', in *OIE Terrestrial Animal Health Manual*, World Organisation for Animal Health, Paris.

— 2016, 'Infectious bursal disease (Gumboro disease)', in *OIE Terrestrial Animal Health Manual*, World Organisation for Animal Health, Paris.

— 2017, 'Infectious bursal disease (Gumboro disease)', in *OIE Terrestrial Animal Health Code*, World Organisation for Animal Health, Paris.

Pantin-Jackwood, MJ & Brown, TP 2003, 'Infectious bursal disease virus and proventriculitis in broiler chickens', *Avian Diseases*, vol. 47, no. 3, pp. 681–90.

Patterson MF, Quinn M, Simpson R & Gilmour A 1995, 'Sensitivity of vegetative pathogens to high hydrostatic pressure treatments in phosphate-buffered saline and foods', *Journal of Food Protection*, vol. 58, pp. 524–9.

Pontes L, Fornells IA, Giongo V, Araujo JRV, Sepulveda A, Villas-Boas M, Bonate CFS & Silva JL 1997, 'Pressure inactivation of animal viruses: potential biotechnological applications', in Heremans K (ed.), *High pressure research in biosciences and biotechnology*, Leuven Univ Press, Leuven, Belgium.

Rendueles, E, Omer, M, Alvseike, O, Alonso-Calleja, C, Capita, R & Prieto, M 2011, 'Microbiological food safety assessment of high hydrostatic pressure processing: a review', *LWT—Food Science and Technology*, vol. 44, no. 5, pp. 1251–60.

Setikaite I, Koutchma T, Patazca E & Parisi E 2009, 'Effects of water activity in model systems on high-pressure inactivation of *Escherichia coli*', *Food and Bioprocess Technology*, vol. 2, pp. 213–21.

Sheen S, Cassidy J, Scullen B, Uknalis J & Sommers C 2015, 'Inactivation of *Salmonella* spp. in ground chicken using high pressure processing', *Food Control*, vol. 57, pp. 41–7.

Soglia, F, Mudalal, S, Babini, E, Di Nunzio, M, Mazzoni, M, Sirri, F, Cavani, C & Petracci, M 2016, 'Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality', *Poultry Science*, vol. 95, no. 3, pp. 651–9.

Stoute, ST, Jackwood, DJ, Sommer-Wagner, SE, Crossley, BM, Woolcock, PR & Charlton, BR 2013, 'Pathogenicity associated with coinfection with very virulent infectious bursal disease and Infectious bursal disease virus strains endemic in the United States', *Journal of Veterinary Diagnostic Investigation*, vol. 25, no. 3, pp. 352–8.

Swayne, DE 2006, 'Microassay for measuring thermal inactivation of H5N1 high pathogenicity avian influenza virus in naturally infected chicken meat', *International Journal of Food Microbiology*, vol. 108, no. 2, pp. 268–71.

Tananuwong, K, Chitsakun, T & Tattiyakul, J 2012, 'Effects of high-pressure processing on inactivation of *Salmonella typhimurium*, eating quality, and microstructure of raw chicken breast fillets', *Journal of Food Science*, vol. 77, no. 11, pp. E321–7.

Tanimura N, Tsukamoto K, Nakamura K, Narita M & Maeda M 1995, 'Association between pathogenicity of IBD virus and viral antigen distribution detected by immunohistochemistry', *Avian Diseases*, vol. 39, pp. 9–20.

Thomas, C, King, DJ & Swayne, DE 2008, 'Thermal inactivation of avian influenza and Newcastle disease viruses in chicken meat', *Journal of Food Protection*, vol. 71, no. 6, pp. 1214–22.

Tian, SM, Ruan, KC, Qian, JF, Shao, GQ & Balny, C 2000, 'Effects of hydrostatic pressure on the structure and biological activity of infectious bursal disease virus', *European Journal of Biochemistry*, vol. 267, no. 14, pp. 4486–94.

Wilkinson N, Kurdziel AS, Langton S, Needs E & Cook N 2001, 'Resistance of poliovirus to inactivation by high hydrostatic pressure', *Innovative Food Science & Emerging Technologies*, vol. 2, pp. 95–8.

Yuste, J, Pla, R & Mor-Mur, M 2000, '*Salmonella enteritidis* and aerobic mesophiles in inoculated poultry sausages manufactured with high-pressure processing', *Letters in Applied Microbiology*, vol. 31, no. 5, pp. 374–7.

References sought by the department:

Abdul, R, Murgia, M, Rodriguez-Palacios, A, Lee, C & Saif, Y 2013, 'Persistence and tissue distribution of infectious bursal disease virus in experimentally infected SPF and commercial broiler chickens', *Avian Diseases*, vol. 57, no. 4, pp. 759–66.

Aini, H, Omar, A, Hair-Bejo, M & Aini, I 2008, 'Comparison of Sybr Green I, ELISA and conventional agarose gel-based PCR in the detection of infectious bursal disease virus', *Microbiology Research*, vol. 163, pp. 556–63.

Alexander, DJ & Manvell, RJ 2004, 'Heat inactivation of Newcastle disease virus (strain Herts 33/56) in artificially infected chicken meat homogenate', *Avian Pathology*, vol. 33, no. 2, pp. 222–5.

Barroso, S, Nico, D, Gomes, D, dos Santos, A, Couceiro, J, de Sousa, C, da Silva, J & de Oliveira, A 2012, 'Mice vaccination with high hydrostatic pressure-inactivated H3N8 virus protects against experimental avian flu', *Procedia in Vaccinology*, vol. 6, pp. 98–105.

Burleson, F, Chambers, T & Wiedbrauk, D 2014, *Virology: a laboratory manual*, Academic Press, Inc. Harcourt Brace Jovanovich Publishers.

Cheftel, J 1995, 'Review: high-pressure, microbial inactivation and food preservation', *Food Science and Technology International*, vol. 1, no. 2-3, pp. 75–90.

Cliche, S, Amiot, J, Avezard, C & Gariepy, C 2003, 'Extraction and characterization of collagen with or without telopeptides from chicken skin', *Poultry Science*, vol. 82, no. 3, pp. 503–9.

Dormitorio, TV, Giambrone, JJ, Guo, K & Hepp, GR 2009, 'Evaluation of field and laboratory protocols used to detect avian influenza viruses in wild aquatic birds', *Poultry Science*, vol. 88, no. 9, pp. 1825–31.

Dumard, CH, Barroso, SP, de Oliveira, GA, Carvalho, CA, Gomes, AM, Couceiro, JN, Ferreira, DF, Nico, D, Oliveira, AC, Silva, JL & Santos, PS 2013, 'Full inactivation of human influenza virus by high hydrostatic pressure preserves virus structure and membrane fusion while conferring protection to mice against infection', *PLoS One*, vol. 8, no. 11, p. e80785.

FAO 2011a, *Guidelines for the control of Campylobacter and Salmonella in chicken meat CAC/GL 78-2011*, Food and Agriculture Organization, Rome.

— 2011b, *Guidelines on the application of general principles of food hygiene to the control of viruses in food CAC/GL 79-2012*, Food and Agriculture Organization, Rome.

Glamoclija, N, Starcevic, M, Janjic J, Ivanovic, J, Boskovic, M, Djordjevic, J, Markovic, R & Baltic, MZ 2015, 'The effect of breed line and age on measurements of pH-value as meat quality parameter in breast muscles (m. pectoralis major) of broiler chickens', *Procedia Food Science*, vol. 5, pp. 89–92.

Hassan, MK, Saif, YM & Shawky, S 1996, 'Comparison between antigen-capture ELISA and conventional methods used for titration of infectious bursal disease virus', *Avian Diseases*, vol. 40, no. 3, pp. 562–6.

Ignjatovic, J 2004, *Very virulent infectious bursal disease virus*, CSIRO Livestock Industries, AAHL, Geelong.

Institute of Food Technologists 2000, 'Kinetics of microbial inactivation for alternative food processing technologies', *Journal of Food Science*, vol. 65, p. s8.

Jackwood, DJ 2004, 'Recent trends in the molecular diagnosis of infectious bursal disease viruses', *Animal Health Research Reviews*, vol. 5, no. 2, pp. 313–6.

Jackwood, DJ & Sommer-Wagner, SE 2010, 'Detection and characterization of infectious bursal disease viruses in broilers at processing', *Preventive Veterinary Medicine*, vol. 97, no. 1, pp. 45–50.

Jaturasitha, S, Srikanchai, T, Kreuzer, M & Wicke, M 2008, 'Differences in carcass and meat characteristics between chicken indigenous to northern Thailand (Black-boned and Thai native) and imported extensive breeds (Bresse and Rhode Island red)', *Poultry Science*, vol. 87, no. 1, pp. 160–9.

Kingsley, DH & Chen, H 2008, 'Aqueous matrix compositions and pH influence feline calicivirus inactivation by high pressure processing', *Journal of Food Protection*, vol. 71, no. 8, pp. 1598–603.

— 2009, 'Influence of pH, salt, and temperature on pressure inactivation of hepatitis A virus', *International Journal of Food Microbiology*, vol. 130, no. 1, pp. 61–4.

Kruk, ZA, Kim, HJ, Kim, YJ, Rutley, DL, Jung, S, Lee, SK & Jo, C 2014, 'Combined effects of high pressure processing and addition of soy sauce and olive oil on safety and quality characteristics of chicken breast meat', *Asian–Australasian Journal of Animal Sciences*, vol. 27, no. 2, pp. 256–65.

Linton, M, McClements, JM & Patterson, MF 1999, 'Inactivation of *Escherichia coli* O157:H7 in orange juice using a combination of high pressure and mild heat', *Journal of Food Protection*, vol. 62, no. 3, pp. 277–9.

Lomniczi, B 1975, 'Thermostability of Newcastle disease virus strains of different virulence', *Archives of Virology*, vol. 47, no. 3, pp. 249–55.

Moses, H, Brandly, C & Jones, E 1947, 'The pH stability of viruses of Newcastle disease and fowl plague', *Science*, vol. 105, no. 2731, pp. 477–9.

OIE 2008, *Manual for diagnostic tests and vaccines for terrestrial animals, 6th edition*, World Organisation for Animal Health, Paris.

Oxen, P & Knorr, D 1993, 'Baroprotective effects of high solute concentrations against inactivation of *Rhodotorula rubra*', *LWT—Food Science and Technology*, vol. 26, no. 3, pp. 220–3.

Quality Control Unit 1997, *Heat inactivation of infectious bursal disease virus strain CS88, CVLS/06/97*, Central Veterinary Laboratory Surrey UK, Surrey.

Reed LJ & Muench H 1938, 'A simple method of estimating fifty per cent endpoints', *American Journal of Epidemiology*, vol. 27, no. 3, pp. 493-7.

Soubies, SM, Courtillon, C, Abed, M, Amelot, M, Keita, A, Broadbent, A, Hartle, S, Kaspers, B & Eterradossi, N 2018, 'Propagation and titration of infectious bursal disease virus, including non-cell-culture-adapted strains, using ex vivo-stimulated chicken bursal cells', *Avian Pathology*, vol. 47, no. 2, pp. 179–88.

Swayne, DE & Beck, JR 2004, 'Heat inactivation of avian influenza and Newcastle disease viruses in egg products', *Avian Pathology*, vol. 33, no. 5, pp. 512–8.

Tian, SM, Qian, JF, Shao, GQ & Ruan, KC 1999, 'High immunogenicity of the pressure-inactivated virus', *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)*, vol. 31, no. 3, pp. 334–6.

USDA 2018, Food Composition Databases. United States Department of Agriculture, Agricultural Research Service, viewed 4 February 2019.

Vindevogel, H, Meulemans, G & Halen, P 1975, 'Maladie de Gumboro. Cinetique des anticorps precipitants et neutralisants', *Annales de médecine vétérinaire*.