



**Australian Government**

**Biosecurity Australia**

**IMPORTATION OF HATCHING (FERTILE)  
DUCK EGGS FROM APPROVED  
COUNTRIES**

**Policy review**

**July 2009**



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## Glossary of Terms and Abbreviations

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ABARE	Australian Bureau of Agriculture and Resource Economics
AI	Avian influenza
AIV	Avian influenza virus
ALOP	Appropriate level of protection
AQIS	Australian Quarantine and Inspection Service
cfu	Colony forming unit
CRC	Co-operative Research Centre
DHV	Duck hepatitis virus
DVE	Duck virus enteritis
GPV	Goose parvovirus
HPNAI	Highly pathogenic notifiable avian influenza
HEPA	High efficiency particulate air
IBD	Infectious bursal disease
LPNAI	Low pathogenic notifiable avian influenza
MDPV	Muscovy duck parvovirus
ND	Newcastle disease
NDV	Newcastle disease virus
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PAQ	Post-arrival quarantine
PEQ	Pre-export quarantine
QAP	Quarantine approved premise
SPS Agreement	WTO Agreement on the Application of the Sanitary and Phytosanitary Measures
vvIBD	Very virulent infectious bursal disease
WTO	World Trade Organization

# Summary

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Quarantine policy exists for the importation into Australia of a variety of egg products and for hatching (fertile) eggs of domestic ducks, hens and turkeys from approved countries. There are three sets of requirements for the importation of hatching eggs—one each for domestic hens, turkeys and ducks. The quarantine requirements for imported hatching eggs of domestic hens and turkeys were revised in August 2004 to reduce the post-arrival quarantine (PAQ) period from 12 to 9 weeks post-hatch.

This policy review is in response to a request from the Torrens Island Avian Quarantine Station Stakeholder Group to reduce the length of the PAQ period for poultry hatched from imported duck eggs from 12 to 9 weeks.

This policy review recommends that testing of the PAQ flock can be carried out at six weeks post-hatch rather than nine weeks. This allows the PAQ period for hatching duck eggs to be shortened from 12 to 9 weeks without increasing the quarantine risks, thus continuing to meet Australia's appropriate level of protection (ALOP).

The review also recommends that:

- vaccination of donor flocks against duck hepatitis virus type I be permitted
- vaccination of donor flocks against Newcastle disease continue to be permitted but restricted to the use of inactivated vaccines
- reference to *Salmonella Pullorum* and *Salmonella Gallinarum* with the requirement for serological testing be removed from the import requirements as ducks are relatively resistant to infection and are not known reservoirs of these agents. The post-arrival bacteriological culture for *Salmonella* spp. of pipped<sup>1</sup> embryos, hatchery waste and ducklings that die in PAQ will continue
- reference to *Salmonella Arizona* and requirements to test specifically for this agent be removed from the import requirements as this disease agent is present in Australia and is not considered to have an adverse impact
- reference to *Riemerella anatipestifer* be removed from the import requirements as this agent is not egg-transmitted and is present in Australia
- testing for *Salmonella Enteritidis* be revised, removing the requirement to test culled ducklings and faeces post-arrival. All other testing requirements remain; that is, pre-export testing of the source flock and post-arrival bacteriological culture for *Salmonella* spp. of pipped embryos, hatchery waste and ducklings that die in PAQ will continue; and
- testing in PAQ for multi-drug resistant *Salmonella Typhimurium* DT104 be introduced. This will be undertaken as part of the post-arrival bacteriological culture for *Salmonella* spp. of hatchery waste, pipped embryos and duckling that die in PAQ.

These conclusions are based on a re-examination of the risks associated with the importation of hatching duck eggs from approved countries.

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<sup>1</sup> Fully grown duck hatchlings that penetrate, but fail to hatch out of, the egg.

# 1 Background

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## 1.1 Introduction

Biosecurity Australia is a unit within the Biosecurity Services Group, part of the Australian Government Department of Agriculture, Fisheries and Forestry. Biosecurity Australia provides recommendations for animal quarantine policy to Australia's Director of Animal and Plant Quarantine.

As there is an existing policy for the importation into Australia of hatching eggs of domestic ducks, Biosecurity Australia has undertaken a review of this policy. Hazards (disease agents) potentially associated with the importation of hatching (fertile) duck eggs were re-evaluated. Risk assessments of hazards meeting criteria based on the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code were undertaken and revised measures are recommended. The scientific literature, existing policies and the recent *Final Generic Import Risk Analysis Report for Chicken Meat* (Biosecurity Australia, 2008)<sup>2</sup> were used as references during the preparation of this review.

A draft policy review was issued to stakeholders in March 2009 (Biosecurity Australia Advice 2009/05 of 13 March 2009) for a 30-day comment period. Comments were received from four stakeholders and these have been considered in finalising the review.

## 1.2 Proposal

This review of the policy for the importation of hatching (fertile) duck eggs has been undertaken in response to a request from the Torrens Island Avian Quarantine Station Stakeholder Group to reconsider the length of the post-arrival quarantine (PAQ) period for poultry hatched from imported eggs. Members of the Group include users of the quarantine station and the Australian Quarantine and Inspection Service (AQIS). The Torrens Island Quarantine Station has a full schedule until 2019.

## 1.3 Scope

This policy review considers the quarantine risks that may be associated with the importation into Australia of hatching eggs from domestic ducks (*Anas* spp. and *Cairina moschata*) from approved sources. Approved sources are the United States of America (USA), the United Kingdom (UK), France, Canada, Netherlands, Germany, Ireland and New Zealand.

To ensure currency of the disease list, hazard identification and hazard refinement were undertaken using the list of diseases notifiable to the OIE and a list of the causative agents of other diseases relevant to the importation of hatching duck eggs. The results of the hazard refinement process are shown in Tables 1 and 2 (see Section 2.3).

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<sup>2</sup> See <http://www.daffa.gov.au/ba/ira/current-animal/chicken-meat> (accessed November 2008)

## **1.4 Australia's quarantine policy for the importation of hatching eggs**

Separate import requirements for hatching eggs of domestic hens, turkeys and ducks were established in 1989. In July 2004, requirements for hatching eggs of domestic hens and turkeys were revised with the post-hatch PAQ period reduced from 12 to 9 weeks. The reduced PAQ period was based on the final round of testing at six weeks of age that allowed sufficient time for test results to be returned before release of the birds from quarantine.

In 2005 and 2006, the import requirements for hatching eggs of hens, turkeys and ducks were amended in light of the global situation with regard to highly pathogenic avian influenza.

Import requirements for hatching eggs of hens and turkeys are available by contacting AQIS or at <http://www.aqis.gov.au>.

## **1.5 Potentially affected Australian industries**

The introduction of an exotic disease could potentially affect several Australian industries or groups. A brief description of each industry is given below.

### **1.5.1 The Australian duck industry**

The commercial duck meat industry is dominated by two producers, one in Victoria and the other in New South Wales. They are the main suppliers to the restaurant, hospitality and supermarket sectors. These companies use imported Pekin duck genetic stock and are partially vertically integrated—breeding, growing and processing the ducks and distributing the finished products. The Australian Poultry Co-operative Research Centre (CRC) estimates the industry to be worth over A\$40 million per annum and growing (Australian Poultry CRC 2008).

The duck egg industry is small and produces for specialist outlets.

### **1.5.2 Commercial chicken meat industry**

The Australian Bureau of Agriculture and Resource Economics (ABARE) forward estimate for poultry meat production in 2007–08 was 847 kilotonnes (ABARE 2007). Chicken meat consumption per person is projected to increase to 39.1 kg per person in 2007–08 (ABARE 2007). Three large integrated companies account for about 80% of chicken meat production and processing (Australian Chicken Meat Federation 2005).

There is significant chicken meat production (both growing and processing) in most states and the industry directly employs approximately 40 000 people. Around 800 individual growers produce about 80% of chicken produced under a contract system, which has been a feature of the industry for the past 30 years (Australian Chicken Meat Federation 2005).

There is a growing export market for Australian breeding stock. Growth of this market, will depend, among other things, on the continued absence of major poultry diseases in the Australian breeder flock.

### **1.5.3 Commercial poultry egg industry**

There are about 420 commercial egg producers in Australia, with a national flock size of around 16.2 million (AECL 2007).

The egg industry in Australia is dominated by egg production from the domestic chicken. However, there are niche markets for duck, goose, quail and pigeon eggs. In contrast with the chicken egg industry, non-chicken eggs are produced by small operators or as a sideline to a meat industry.

Domestic retail sales of shell eggs were estimated at A\$266 million between July 2006 and July 2007. The value of exports (shell egg and egg products) was A\$2.264 million in 2005 (AECL 2007).

### **1.5.4 Other groups potentially affected**

- turkey industry
- native birds and the environment
- game birds, e.g. pheasant, guinea fowl
- pigeons
- ratite industry
- aviculture community
- zoos.

## 2 Risk assessment and risk management options

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### 2.1 Overview

Biosecurity Australia is responsible for developing and reviewing quarantine policy for the import of animals and plants and their products. It does this through a science-based risk analysis process. According to the OIE, a risk analysis comprises hazard identification, risk assessment, risk management and risk communication. At the completion of the process, Biosecurity Australia makes a recommendation for a policy determination to Australia's Director of Animal and Plant Quarantine. This determination is taken into account by AQIS when considering applications to import.

Australia's science-based risk analysis process is consistent with Australian Government policy and Australia's rights and obligations under the Agreement on the Application of the Sanitary and Phytosanitary Measures (SPS Agreement).

Australia has a long-standing conservative approach to quarantine risk. The level of risk Australia is prepared to accept is known as Australia's appropriate level of protection (ALOP) and is expressed as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Australia has a consistent approach to addressing risks. Those risks that are very low or less meet Australia's ALOP and no risk management measures are required. For those quarantine risks that exceed Australia's ALOP, risk management measures are recommended to reduce the level of risk in order to achieve the ALOP.

### 2.2 Hazard identification

Hazard identification is defined in the OIE Terrestrial Animal Health Code 2008 as the process of identifying the pathogenic agents that could potentially produce adverse consequences associated with the importation of a commodity. Hazard identification is a classification step, identifying pathogenic agents as potential hazards or not.

To be identified as a potential hazard in this review, a pathogenic agent:

- should be appropriate to the animal species to be imported, or from which the commodity is derived
- should be capable of producing adverse consequences in the importing country
- may be present in the exporting country
- should not be present in the importing country. If present, the pathogenic agent should be associated with a notifiable disease, and be subject to an official control or eradication program.

In this review, hazard identification was initiated by generating a preliminary list of potential pathogenic agents or 'potential hazards'. The list consisted of pathogenic agents associated with each of the OIE listed diseases, and other diseases relevant to the importation of hatching

duck eggs from an international source. This list was refined by applying the criteria stated above to each disease agent.

## 2.3 Hazard list – identification and refinement

A list of potential hazards was compiled by reference to Biosecurity Australia policy documents, the OIE listed diseases and scientific literature which refer to disease agents of relevance to ducks. Together with details of the reasons on whether to retain agents for risk assessment, the potential hazard list is set out in Tables 1 and 2. Of the disease agents considered as being potentially of quarantine concern, 12 were retained for further risk assessment and are presented in Sections 2.4 and 2.5 under the following headings:

- OIE listed avian disease agents and
- Non-OIE listed avian disease agents.

**Table 1: OIE listed disease agents**

Disease agent	Ducks Infected	Other Poultry Affected	Egg-borne transmission <sup>1</sup>	Adverse impact <sup>2</sup>	Present in Australia	Retain for risk assessment
Notifiable avian Influenza virus	yes	all	yes <sup>3</sup>	yes	no	yes
Newcastle disease virus	yes	all	yes <sup>4</sup>	yes	no <sup>5</sup>	yes
Duck hepatitis virus type I	yes	no	no <sup>6</sup>	yes	no	yes
Duck hepatitis virus type II (duck astrovirus)	yes	no	no <sup>6</sup>	yes	no	yes
Duck hepatitis virus type III	yes	no	no <sup>6</sup>	yes	no	yes
Duck virus enteritis	yes	geese	yes	yes	no	yes
Infectious bursal disease virus (very virulent and exotic variant)	yes	chicken	yes	yes	no	yes
<i>Mycoplasma gallisepticum</i>	yes	chickens turkeys	yes <sup>7</sup>	yes	yes	no
<i>Chlamydophila psittaci</i>	yes	all	yes	yes	yes	no
<i>Pasteurella multocida</i>	yes	chickens turkeys	no	yes	yes	no
<i>Salmonella Pullorum</i> <i>Salmonella Gallinarum</i>	yes	chickens turkeys	yes <sup>7</sup>	yes	no <sup>8</sup>	yes

**Table 2: Other disease agents**

Disease agent	Ducks Infected	Other Poultry Affected	Egg-borne transmission <sup>1</sup>	Adverse impact <sup>2</sup>	Present in Australia	Retain for risk assessment
Avian metapneumovirus	yes	chicken turkeys	no	yes	no	no
<i>Salmonella</i> Enteritidis	yes	chickens	yes <sup>7</sup>	yes	no <sup>9</sup>	yes
<i>Salmonella</i> Typhimurium DT104	yes	all	yes <sup>10</sup>	yes	no	yes
Antibiotic resistant <i>Campylobacter jejuni</i>	yes	all	yes	yes	yes	no
<i>Riemerella anatipestifer</i>	yes	geese turkeys	no	yes	yes <sup>11</sup>	no
<i>Ornithobacterium rhinotracheale</i>	yes	all	no	no	no	no
<i>Salmonella</i> Arizona	yes	chicken turkeys	yes	no <sup>12</sup>	yes <sup>13</sup>	no
Reticuloendotheliosis virus	yes	chicken	yes	yes	yes	no
<i>Mycoplasma synoviae</i>	yes	chickens turkeys	yes <sup>7</sup>	yes	yes	no
<i>Mycobacterium avium-intracellulare</i>	yes	all	yes <sup>7</sup>	yes	yes	no
Avian paramyxoviruses 2,3	no	turkeys chickens	yes	yes	no	no
West Nile virus	yes	all	no	yes	no	no
Goose parvovirus	Muscovy ducks only	geese	yes	yes	no	yes
Muscovy duck parvovirus	Muscovy ducks only	no	yes	yes	no	yes
Reovirus of Muscovy ducks	Muscovy ducks only	mule ducks <sup>14</sup>	yes	yes	no	yes
Duck circovirus	yes	no	yes <sup>15</sup>	no	? <sup>16</sup>	no

<sup>1</sup>. Egg-borne transmission: transmission from the infected dam to newly hatched offspring by means of the fertile egg. Some disease agents are as a result of shedding into the egg prior to the addition of the shell and membranes; others penetrate or are carried on the shell surface

<sup>2</sup>. Adverse impact: the pathogenic agent (or a clearly identified strain of the pathogenic agent) could potentially produce adverse consequences in susceptible humans or animals in importing countries

<sup>3</sup>. Avian influenza virus has been isolated from the contents and surface of chicken eggs

<sup>4</sup>. Virus isolated from chicken egg contents—trans-ovarian transmission to ducklings unknown

<sup>5</sup>. Virulent Newcastle disease (Australian origin) has occurred in Australia, but has been eradicated

<sup>6</sup>. Although egg transmission is presumed not to take place, the virus is capable of survival for long periods and Biosecurity Australia considers that there is potential that duck hepatitis virus could enter PAQ via shell contamination of imported hatching duck eggs

<sup>7</sup>. Trans-ovarian transmission in chickens—unknown in ducks

<sup>8</sup>. Eradicated from commercial flocks

<sup>9</sup>. Subtypes of concern, for example phage types 4, 8 and 13a, not present in Australia

<sup>10</sup>. Transmission through penetration of salmonellae into or through the shell and shell membranes

<sup>11</sup>. No evidence that local strains are different to overseas strains

<sup>12</sup>. Not OIE listed and not notifiable in Australia

<sup>13</sup>. Isolated in Australia from reptiles (Bell 1986)

<sup>14</sup>. Muscovy—common duck hybrids

<sup>15</sup>. Based on chicken circoviruses such as chicken anaemia virus

<sup>16</sup>. Unknown—may be widespread

Measures for *Riemerella anatipestifer* and *Salmonella* Arizona are included in the 2006 import requirements but as they have been assessed to have negligible adverse impact and are present in Australia, they were removed from further assessment and from the recommended quarantine requirements (Appendix 5.1).

**Table 3: Disease agents retained for risk assessment**

Duck hepatitis virus type I
Duck hepatitis virus type II
Duck hepatitis virus type III
Duck virus enteritis
Newcastle disease virus
Notifiable avian influenza virus
Infectious bursal disease virus
<i>Salmonella</i> Pullorum and <i>S. Gallinarum</i>
<i>Salmonella</i> Enteritidis
<i>Salmonella</i> Typhimurium DT104
Goose parvovirus
Muscovy duck parvovirus
Reovirus of Muscovy ducks

## 2.4 Assessment of OIE listed avian disease agents

### 2.4.1 Duck hepatitis virus

Hepatitis in ducks can be caused by at least three different viruses. The more common and internationally widespread is duck hepatitis virus (DHV) type I, an enterovirus of the family *Picornaviridae* which causes an acute, highly lethal, contagious infection in ducklings less than six weeks of age. Disease does not occur in older birds. It has been suggested that DHV type I should be classified as a separate genus within *Picornaviridae* (Kim *et al.* 2006). DHV type I infection is most often referred to as duck virus hepatitis.

DHV types II and III are astroviruses which cause similar but milder disease in ducklings between six and ten weeks of age (Knowles 2006).

Diagnosis of hepatitis in ducklings is based on the characteristic disease pattern in the flock, gross pathological changes, virus isolation and identification, and reproduction of the disease in susceptible ducklings. Immunological tests are not used extensively for the routine identification of DHV type I, II or III infections.

A new serotype of DHV named N-DHV has been reported to cause high mortality with characteristic liver lesions (Tseng and Tsai 2007). This virus was recovered from both mule ducklings and goslings (OIE 2008).

#### **2.4.1.1 Duck hepatitis virus I**

Classical duck virus hepatitis (DHV type I) is endemic in most important duck-growing regions of the world, including Denmark, Ireland, Korea, France, Malaysia, the UK, the USA, China and Vietnam. Ducks are the only known natural hosts of DHV type I virus (Calnek 1993). This agent has not been reported in Australia.

Under field conditions DHV type I spreads rapidly through a flock causing an acute (clinical signs within one to two hours are possible), often fatal disease in ducklings less than six weeks of age. The clinical disease is characterised by lethargy, ataxia, opisthotonos and death.

In experimental infections of ducklings one to seven days old the incubation period for DHV type I can be less than 24 hours with deaths generally occurring within four days of infection. However, the OIE defines the incubation period of DHV as seven days for disease control purposes (OIE 2008).

Morbidity is generally 100%. The mortality rate varies with the age of the bird but may reach 95% in ducklings less than one week old and up to 50% in three week old ducklings. Practically all mortality in a flock will occur within three to four days. Morbidity and mortality is reported to be low or negligible in ducklings four to five weeks of age (Hwang 1970; Woolcock 2003). Sub-clinical infections occur in ducklings infected after four to six weeks of age (Hwang 1970; Calnek 1993). Ducks that have recovered from the disease excrete virus in their faeces for up to eight weeks post-infection and develop protective immunity (Woolcock and Fabricant 1997; Woolcock 2003).

The natural route of transmission is via ingestion of contaminated feed or water with the portal of entry probably the pharynx or upper respiratory tract (Toth and Norcross 1981).

#### **2.4.1.2 Duck hepatitis virus II and III**

DHV types II and III have been reported in ducklings immune to DHV type I and have only been reported from the UK and the USA respectively (Toth 1969; Gough *et al.* 1985; Gough 1986). Neither agent has been reported in Australia.

Ducklings infected with DHV type II show clinical and pathological signs similar to DHV type I but with lower rates of mortality. Affected birds may show signs of polydipsia and usually die within one to two hours of clinical signs appearing. DHV type II is excreted in the faeces and appears to spread horizontally by contact. Survivors excrete the virus for at least one week after infection, and are immune to further infection (Gough and Stuart 1993). Mature ducks are reported to be refractory to disease (Woolcock and Fabricant 1997).

DHV type III is less severe than DHV type I, with a mortality rate of up to 20% in ducklings immune to DHV type I. Clinical signs are similar to those seen with type I infections (Haider and Calnek 1979). There have been no reports in the literature of DHV type III outbreaks since the 1970s.

#### **2.4.1.3 Vaccination**

Live attenuated DHV type I vaccines are used before the start of egg laying to immunise breeder ducks or to actively immunize susceptible ducklings. Breeder ducks, primed with live attenuated vaccine or previously exposed to live DHV type I, may also be vaccinated using an inactivated DHV type I vaccine. Well-vaccinated breeder ducks may transmit passive immunity in eggs for eight to nine months (Woolcock 2003).

In ducklings from vaccinated flocks, maternal immunity wanes by two to three weeks of age (Calnek 1993; Liu and Higgins 1990). Duck breeder flocks vaccinated before laying commences will not show an immunological response to live virus challenge as adults (Woolcock 1991).

An experimental live attenuated DHV type II vaccine was shown to protect ducklings but was not produced commercially (Gough and Stuart 1993).

DHV type III infections have been controlled by the use of attenuated live virus vaccines given to breeder ducks (Haider and Calnek 1979).

#### **2.4.1.4 Transmission in eggs**

Egg-borne transmission has not been reported for DHV types I, II and III. However, DHV type I is capable of survival for long periods under usual environmental conditions (Woolcock 2003) so egg surface contamination could cause infection of hatching ducks.

#### **2.4.1.5 Conclusion**

Australia's 2006 import requirements state that the source flock is to be tested for DHV type I before egg collection and that the flock show no evidence of clinical signs of disease for 90 days prior to egg collection. If infection is present, it is unlikely to be detected in the source flock as clinical signs in all forms of hepatitis are seen only in young ducklings. Hatching eggs are required to undergo disinfection after collection and again on arrival in PAQ.

Diagnosis of DHV in PAQ relies mainly on the sudden and significant mortality the virus causes in susceptible ducklings. Investigation of any mortality in the PAQ flock would detect infection within the first three to four weeks of hatching (Woolcock, P.R., *pers comm.* 2004).

The 2006 requirements stipulate serological testing of the PAQ flock at nine weeks of age for DHV. An immune response to exposure to virus post-hatch would be expected to occur within five weeks. Consequently, the recommended reduction in the length of PAQ and serological testing at six weeks post-hatch would be expected to detect an antibody response if the virus was present.

In conjunction with the recommended reduction in the PAQ period, a further change is recommended with respect to DHV type I. As DHV type I is endemic in most important duck-growing regions in the world, and vaccination for the disease is performed routinely, it is recommended that the importation of fertile eggs from flocks vaccinated with DHV type I be permitted. Vaccination must occur more than 10 weeks before the commencement of pre-egg collection testing. Accordingly serological testing of vaccinated donor flocks before egg collection would not be required.

Transovarian transmission of DHV virus is presumed to not occur and any residual virus contamination of the egg surface would be expected to be removed by disinfection of eggs pre-export and in PAQ.

Recognition of DHV in PAQ would still rely on the investigation of morbidity and mortalities. In this situation, if hatched ducklings are exposed to virus in PAQ, infection would occur when maternal antibodies have waned after three weeks post-hatch. By this time, ducklings are less susceptible to infection but mortality would still be significant and would be investigated—natural age resistance only becomes fully protective between four and six weeks of age. Antibody production after exposure at three weeks of age would be expected to

evident from five weeks of age so that serological testing of ducklings at six weeks would provide an additional assurance of freedom.

### **2.4.2 Duck virus enteritis**

Duck virus enteritis (DVE), also known as duck plague, is caused by an Alpha herpesvirus. DVE is a contagious disease of ducks, geese and swans, usually associated with acute signs, high morbidity and mortality in naïve flocks (Sandhu and Shawky 2003). Outbreaks may cause serious economic losses due to decreased egg production in susceptible mature breeders, and increased mortality and carcass condemnation in ducklings. DVE is considered a threat to wild and migratory waterfowl populations (Shawky and Schat 2002).

DVE has been reported in the USA, Canada, Europe, Asia and Brazil but not Australia (Hansen and Gough 2007).

Susceptibility to experimental infection varies between species; mallards are reported to be more resistant to lethal infection than other species (Sandhu and Leibovitz 1997) while Muscovies are among the more susceptible species (Davison *et al.* 1993). Major outbreaks of DVE have been reported in wild migratory waterfowl in the USA and serious outbreaks have been reported in zoos and game farm flocks (Friend 1999).

Outbreaks in domestic ducks are frequently associated with access to open bodies of water, cohabited by free-flying waterfowl (Sandhu and Shawky 2003). DVE has been reported in domestic ducks of all ages, ranging from seven days to mature breeders. The incubation period ranges from three to seven days in domestic ducks (Sandhu and Shawky 2003). Severity of infection varies with the species, age and sex of the affected birds and the virulence of the virus. High, persistent flock mortality is often the first sign of infection. As DVE progresses in adult birds, photophobia, inappetence, extreme thirst and watery diarrhoea appear. Younger ducklings show dehydration, weight loss, cyanosis of the beak and often a bloodstained vent (Leibovitz 1991). The mortality rate in susceptible flocks ranges from 5% to 100% (Sandhu and Leibovitz 1997).

Horizontal spread is probably the principal mechanism of transmission. The virus is shed in faeces and in oral-respiratory secretions, and is transmitted by direct contact between infected and susceptible birds, or indirectly by contact with a contaminated environment. Water, on which waterfowl depend for feeding, drinking and body support, appears to be the natural means of virus transmission (Sandhu and Leibovitz 1997).

In chronically infected partially immune domestic flocks, only occasional deaths occur. Recovered ducks may be immune to re-infection and become carriers, shedding virus periodically in faeces (Sandhu and Shawky 2003).

The virus also establishes an asymptomatic carrier state in naturally infected waterfowl and is detectable in cloacal swabs and samples of oral lesions during periods of intermittent virus shedding. Cloacal swabs from naturally infected black ducks and Canada geese yield virus for up to four years post infection (Burgess *et al.* 1979). It is not known if the amount shed is sufficient to infect birds. Healthy birds may shed virus and show no detectable serum neutralising antibody (Burgess and Yuill 1983).

It has been shown that birds with a low serological response to vaccination are protected against challenge (Kulkarni *et al.* 1998; Butterfield and Dardiri 1969). Serum neutralisation tests have been used to monitor exposure to DVE in wildfowl (Woolcock 1998). More recently, polymerase chain reaction (PCR) assays have been developed which are more

sensitive than virus isolation techniques for detecting DVE (Plummer *et al.* 1998; Hansen *et al.* 1999; Pritchard *et al.* 1999; Hansen *et al.* 2000).

#### **2.4.2.1 Vaccination**

Chicken-embryo-adapted modified live virus vaccines are available to control DVE in domestic ducks (Toth 1971; Shawky and Sandhu 1997). Vaccine virus does not spread from vaccinated to unvaccinated birds, but it is not known if shedding of virulent virus is reduced or if latency is established in vaccinated birds. Maternal immunity has been reported in ducklings but it declines rapidly. In one study most ducklings were susceptible to challenge at 13 days of age (Sandhu and Shawky 2003; Toth 1970).

#### **2.4.2.2 Transmission in eggs**

Transovarian transmission of virus has been demonstrated in infected carrier ducks. Decreased fertility and hatchability and persistent excretion of virus by hatchlings provide evidence for vertical transmission (Burgess and Yuill 1983).

#### **2.4.2.3 Conclusion**

Australia's 2006 import requirements state that there should be no evidence of illness in the source flock from 90 days prior to egg collection. The source flock is tested by serology for DVE before egg collection. DVE is egg-transmitted and if it occurs, infection is likely to spread rapidly within the hatchery. As the 2006 import requirements stipulate investigation of any mortality within the PAQ flock, it is likely that the disease, if present, would be detected shortly after hatching.

The 2006 import conditions require serological testing of the PAQ flock for DVE at nine weeks of age. It is assumed an immune response would occur within five weeks in ducklings with no maternal antibody protection if infected. Consequently, serological testing at six weeks would be expected to detect any antibody response if virus was present. Negative results of testing at six weeks of age, rather than nine weeks provide an equivalent assurance that DVE is not present in the imported birds.

Virus isolation from cloacal swabs from a sample of PAQ flock at six weeks of age was considered unsuitable by Biosecurity Australia as the virus, if present, may not be shed at the time of sampling. The 2006 import requirements do not include virus isolation.

The 2006 requirements that the source flocks not be vaccinated are considered adequate because the effects of vaccination on the re-infection of immunised birds, their carrier status, and vertical transmission of the virus are not clear.

### **2.4.3 Newcastle disease virus**

Newcastle disease virus (NDV) has a worldwide distribution. Strains of NDV vary greatly in their virulence and tissue tropism and, in susceptible birds, infection induces a wide range of clinical signs and pathological lesions. Based on the severity of the disease produced in infected chickens, NDV strains are broadly classified as velogenic (highly virulent), mesogenic (moderately virulent) and lentogenic (mildly virulent) (Alexander 2003).

Newcastle disease (ND) is an OIE listed disease. Although outbreaks in Australia have been recorded, Australia is currently considered free of virulent ND, with vaccination, in accordance with the OIE definition of a ND-free country.

Natural hosts of NDV are domestic poultry, including chickens, turkeys, ducks, geese, pigeons, quail, pheasants, guinea fowl and ostriches, and many species of caged and wild birds (Alexander 2000). Susceptibility varies between species, with chickens the most likely to show clinical ND and water birds, including ducks, the least likely to be affected clinically (Kaleta and Baldauf 1988). Outbreaks of severe disease in ducks due to NDV have been recorded, but ducks are usually regarded as clinically resistant even to strains that are pathogenic to chickens (Alexander 2003).

ND is highly contagious and transmission of virus is frequently by direct contact with diseased or carrier birds. During the course of infection, large amounts of virus are excreted in faeces and in the expired air for at least one day before clinical signs become apparent (Sinha *et al.* 1954). Birds can be infected by aerosol inhalation or by ingestion, or from fomites such as chicken crates, egg flats, contaminated feed, trucks, dust, humans, other animals, feathers and clothing. Fomites are important in the spread of the disease in an outbreak (Lancaster and Alexander 1975; Alexander 2000).

The incubation period depends on the dose of the virus, route of exposure and the species exposed. In the chicken, the incubation period ranges from 2 to 15 days (generally five to six days) and antibodies to NDV develop within six to ten days of infection (Alexander 1997). A long-term carrier state has been postulated for both lentogenic and velogenic NDV (Heuschele and Easterday 1970).

Humans may also occasionally become infected with NDV; infections associated with NDV have been reported in workers in laboratories and poultry processing plants (Chang 1981).

#### **2.4.3.1 Vaccination**

Both live and inactivated (killed) vaccines are used in the control of ND. Vaccinated chickens are protected from clinical disease but virulent virus may still replicate, be excreted and be present in tissues and organs of clinically healthy immune birds (Asplin 1952; Utterback and Schwartz 1973). Therefore, in a country where regular vaccination is practised, virulent viruses may be circulating without causing clinical disease. It is accepted that the level of infection would be lower than in non-vaccinated, infected flocks.

NDV is readily transmissible, especially among birds kept in large groups. Therefore, it can be assumed that, if NDV is present within an unvaccinated flock of ducks, a large proportion of the flock will be infected.

#### **2.4.3.2 Transmission in eggs**

Little is known about the transmission of ND viruses in eggs. Velogenic NDV has been sporadically identified in eggs of infected hens based on recovery of viruses from hatched chicks or from cell cultures prepared from embryos of infected hens (Capua *et al.* 1993; Chen and Wang 2002). NDV has been demonstrated in and on eggs (Lancaster 1963; Williams and Dillard 1968) and in the reproductive tract of hens (Biswal and Morrill 1954). There is some evidence to suggest that egg transmission may occur (Hofstad 1949; Bivins *et al.* 1950; Zagar and Pomeroy 1950; French *et al.* 1967; Collins *et al.* 1993). Egg transmission of virulent strains in chickens has not been considered to be of epidemiological significance as infected

birds quickly ceased laying and infected embryos died (Beard and Hanson 1984). However, more recent studies have shown that immune hens challenged with virulent NDV may lay contaminated eggs. While no virus was isolated from the shell in these studies, challenge virus was isolated from the albumen of 1 of 187 eggs produced within two weeks of challenge.<sup>3</sup>

While there are no data available on NDV transmission in duck eggs, it is assumed that transmission would be similar to that in hen eggs. As ducks do not show signs of clinical ND, it is considered that they would be unlikely to suffer a drop in egg production as a result of infection with the virus. Therefore, NDV could be present in, or on, a proportion of eggs laid by ducks in an infected flock.

#### **2.4.3.3 Conclusion**

The 2006 import requirements allow vaccination of duck donor flocks—this is consistent with the measures for ND in the chicken hatching egg conditions.

In unvaccinated duck donor flocks the combination of serology and virus isolation provides assurance that the flock is free of infection.

In line with the requirement for eggs from vaccinated donor flocks in the chicken hatching egg conditions, it is recommended that paired blood samples are collected from the source flock. The first samples to be collected not more than 21 days before the first day of egg collection and the repeat test not less than 14 days after the last day of egg collection. A rising titre would indicate a disease challenge with associated higher likelihood of a virulent virus being present in or on the fertile eggs.

The use of live vaccines, either in combination with inactivated vaccines or by themselves, could lead to positive virus isolation results. In order that the risk management step of testing of vaccinated duck donor flocks by virus isolation is not compromised, it is recommended that vaccination of duck donor flocks be restricted to the use of inactivated vaccines.

Given the highly contagious nature and short incubation period of ND, the virus would spread rapidly among the PAQ flock and sentinel chickens<sup>4</sup>. If present, NDV would be readily detected within the six week post-hatch testing period with virus isolation from duckling in the PAQ duck flock and serology of sentinel chickens. These measures are considered to provide an equivalent level of risk management to testing at nine weeks.

#### **2.4.4 Avian Influenza virus**

Avian influenza viruses (AIV) circulate in wild and domestic birds. Most AIV are of low pathogenicity, producing either subclinical disease or mild respiratory or reproductive disease in domestic and wild birds. However, notifiable forms of AIV do exist which are either highly pathogenic notifiable avian influenza (HPNAI) or low pathogenicity notifiable avian influenza (LPNAI) that have the potential to mutate to highly pathogenic forms.

HPNAI is a highly contagious systemic disease of poultry that causes high mortality in chickens. While LPNAI viruses circulate widely in wild bird populations, HPNAI viruses do

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<sup>3</sup> Unpublished report, Australian Animal Health Laboratory 2002

<sup>4</sup> SPF (Specific Pathogen Free) chickens placed at a ratio of 1:50 amongst the PAQ duck flock.

not have a recognised wild bird reservoir. HPNAI viruses have been documented to arise from mutations in LPNAI viruses, with mutations probably occurring within domestic poultry populations (Garcia *et al.* 1996; Swayne and Suarez 2000). Migratory birds are implicated in the recent westward spread of the highly pathogenic H5N1 virus from China to a number of countries in central and west Asia, Europe, and Northern Africa. Outbreaks of notifiable avian influenza in Australia have been recorded, most recently in 1997 (Swayne and Suarez 2000), and all were resolved by a stamping out policy. Australia is currently free of HPNAI and LPNAI.

The incubation period for AI may vary from a few hours to three days in individual birds, or up to 14 days in a flock (Easterday *et al.* 1997). The incubation period depends on the dose of the virus, route of exposure and species exposed. Ducks frequently do not show clinical signs; an exception being during recent H5N1 outbreaks (Sturm-Ramirez *et al.* 2004). Ducks can shed virus for up to 30 days (Swayne and Halvorson 2003).

Serological tests are used to demonstrate the presence of antibodies that may be detected as early as seven to ten days after infection. However, there is considerable variation in the immune response among the various avian species (Easterday *et al.* 1997). For example, antibodies may be undetectable in ducks known to have been infected (Swayne and Halvorson 2003).

#### **2.4.4.1 Transmission in eggs**

AIV can be present in or on the surface of eggs laid by naturally-infected hens (Easterday *et al.* 1997). H5N2 virus was isolated from the albumen, the yolk and the shell surface of infertile eggs laid by infected hens during the 1983-84 outbreak of HPNAI in Pennsylvania, USA (Cappucci, Jr. *et al.* 1985). Data reported in the same study indicated that the virus can survive for at least several days in the albumen and yolk of eggs stored at 10-18°C. In an experimental study using HPNAI to infect mature white leghorn hens, the virus was also recovered from 12 of 14 eggs laid on the third day post-inoculation (Beard *et al.* 1984).

While there is generally a large drop in egg production in flocks experiencing an outbreak of HPNAI, virus has been isolated from clinically unaffected birds during an outbreak (Cappucci, Jr. *et al.* 1985). Most eggs laid during an outbreak of HPNAI were of market quality; although, approximately 10% were thin or soft shelled or abnormally small (Cappucci, Jr. *et al.* 1985).

There are no available data on egg transmission of HPNAI or LPNAI in duck eggs. However, given that HPNAI causes a systemic disease in both chickens and ducks with egg transmission in hen eggs, it is highly probable that HPNAI and LPNAI would be present in duck eggs.

#### **2.4.4.2 Conclusion**

The 2006 import requirements state that the country of export is to be officially free from HPNAI in poultry as defined by the OIE Terrestrial Animal Health Code, and there is no evidence of illness in the source flock for 90 days prior to egg collection, and that the source flock is not vaccinated against AI.

In addition, the requirement for the source flock to be tested free of AI before and after egg collection provides a high level of confidence regarding the AI-free status of the flock.

Disinfection of hatching eggs after collection, and again on arrival in PAQ, reduces the likelihood of shell contamination.

Given the highly contagious nature and short incubation period of AI, the virus would spread rapidly among the PAQ flock and sentinel chickens<sup>5</sup>. If present, AI would be readily detected within the six week post-hatch testing period through virus isolation from the PAQ duck flock and serology of sentinel chickens, and this is considered to provide an equivalent level of risk management to testing at nine weeks.

#### **2.4.5 Infectious bursal disease virus**

Infectious bursal disease (IBD) is an acute, contagious viral infection of chickens, which causes immunosuppression in young chicks, and disease and mortality in three to six week-old chickens (van den Berg *et al.* 2000; Lukert and Saif 2003). IBD virus is distributed worldwide and occurs in all major poultry producing areas, with the probable exception of New Zealand (Thomson 2008). IBD viruses can be classified according to virulence, as attenuated (vaccine strains), classical virulent, variant and very virulent (vvIBD virus, sometimes known as hypervirulent) (van den Berg *et al.* 2000). Both classic and antigenic variant strains exist in Australia, but these can be genetically differentiated from overseas classic, variant and very virulent strains (Sapats and Ignjatovic 2000; Ignjatovic and Sapats 2002). Two serotypes exist—IBD virus serotype 1 causes clinical disease in chickens but has also been isolated from turkeys; IBD virus serotype 2 is not known to cause disease and has been isolated from turkeys and chickens, with serological evidence of infection in other species of birds, including ducks (Lukert and Saif 2003).

Vaccines currently used in Australia do not protect against variant IBD virus strains from North America (Ignjatovic *et al.* 2001). Very virulent strains are exotic to Australia but are found in Europe, Asia and South America (van den Berg *et al.* 1991; Lin *et al.* 1993; Di Fabio *et al.* 1999; Ikuta *et al.* 2001). Very virulent IBD has recently been reported in the United States (Stoute *et al.* 2009).

In ducks, a serotype 1 IBD virus has been isolated from faeces of clinically healthy adult ducks, but the significance of the isolate was uncertain (McFerran *et al.* 1980). Experimental inoculation of ducks with IBD virus caused sero-conversion but no clinical signs or pathology and the virus could not be re-isolated from the inoculated ducks (Yamada *et al.* 1982).

Gilchrist (2005) and Biosecurity New Zealand (2006) cite two Chinese papers that confirm sero-conversion and again report isolation of serotype 1 IBD virus from sero-positive ducks. The import risk analysis on cooked duck meat importation to New Zealand (Biosecurity New Zealand 2006) states that susceptible ducks in-contact with both experimentally inoculated ducks and SPF chickens sero-convert, as tested by a serum neutralisation test, and that IBD virus could be isolated from pooled cloacal swabs, confirming viral replication.

IBD virus is highly contagious and is able to persist in the environment between outbreaks. The main route of transmission in chickens is the faecal-oral route and the virus can survive for prolonged periods in faeces and bedding (Benton *et al.* 1967). Mechanical transmission of virus can occur via faecal contamination of fomites (van den Berg *et al.* 2000).

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<sup>5</sup> SPF (Specific Pathogen Free) chickens placed at a ratio of 1:50 amongst the PAQ duck flock.

### **2.4.5.1 Transmission in eggs**

There is no evidence that IBD virus is transmitted vertically in any species (van den Berg *et al.* 2000; Lukert and Saif 2003). An unpublished experimental trial in chickens showed that IBD virus was not isolated from the yolk, albumen or shell of eggs laid by non-vaccinated 24-week old hens challenged with vvIBD virus, despite the presence of virus in cloacal swabs for two to three days after inoculation<sup>6</sup>.

### **2.4.5.2 Conclusion**

Any IBD infection of duck source breeder flocks would be inapparent. Although vertical transmission of the virus has an extremely low likelihood, faecal contamination of the surface of the shell of a duck egg with virus could occur if the donor flock were infected.

The 2006 import requirements stipulate that hatching eggs be disinfected after collection and again on arrival in PAQ. Nonetheless, IBD virus is extremely hardy and may survive disinfection. In the event that the external shell of imported eggs is contaminated, this could lead to infection of ducklings shortly after hatch. Given there is the potential that exotic variant or very virulent IBD virus could enter PAQ via the importation of hatching duck eggs, the requirement to test sentinel chickens for IBD virus in PAQ should be retained.

IBD virus is hardy and highly contagious. It has a short incubation period and would spread quickly to the sentinel chicken flock. In sentinel birds with no maternal antibody, IBD would be readily detected within the six week post-hatch testing period.

## **2.4.6 *Salmonella Pullorum* and *Salmonella Gallinarum***

Pullorum disease (*S. Pullorum*) and fowl typhoid (*S. Gallinarum*) are septicaemic bacterial diseases of chickens, turkeys and pheasants. These diseases are commonly discussed together because of their similarity in terms of epidemiology and management (Shivaprasad 2000; OIE 2004). They are distinguished from the remainder of the salmonellae, in that they are host-adapted and highly pathogenic for avian species, but are considered to pose little zoonotic risk (OIE 2004).

Both *S. Pullorum* and *S. Gallinarum* are OIE listed disease agents and have been eradicated from commercial poultry flocks in Australia as they have been in the USA, Canada, Japan and most countries in Western Europe (Rabsch *et al.* 2000; Shivaprasad 2003). The Australian Salmonella Reference Laboratory has not recorded isolation of *S. Pullorum* from any Australian source since 1992 (Davos *et al.* 2005) and *S. Gallinarum* was last reported in Australia in 1952 (Animal Health Australia 2001).

Chickens are the natural hosts for both *S. Pullorum* and *S. Gallinarum*. Naturally occurring infections with *S. Pullorum* and *S. Gallinarum* have been reported in ducks (Buxton 1957; Chute and Gershman 1963; Snoeyenbos 1991; Barrow *et al.* 1999); however, ducks appear to be quite resistant to the disease agents (Chute and Gershman 1963; Buchholz and Fairbrother 1992; Barrow *et al.* 1999).

An experimental study has reported that when ten day-old mallard ducklings were inoculated with *S. Pullorum* either orally ( $10^5$  to  $10^{10}$  colony forming units (cfu)/ml) or intravenously ( $10^3$  to  $10^8$  cfu/ml), neither mortality nor morbidity was observed (Buchholz and Fairbrother 1992). In addition, viable *S. Pullorum* was isolated from livers at two weeks post-inoculation

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<sup>6</sup> Unpublished report, Australian Animal Health Laboratory 2003

but not at three weeks post-inoculation. All histopathological examinations of lungs, heart, liver, kidneys, pancreas and spleen were normal. It was concluded that mallards experience a short, subclinical infection that is resolved without lasting tissue damage.

Ducks are very resistant to experimental infection with *S. Gallinarum* (Barrow *et al.* 1999). A group of day-old ducks inoculated orally with  $3 \times 10^8$  cfu in 0.3 ml of *S. Gallinarum* exhibited neither mortality nor morbidity during the three week experimental period. Post-mortem examination of livers and spleens three weeks after inoculation revealed no abnormalities. In addition, no growth of *S. Gallinarum* was observed by culturing liver swabs on MacConkey agar, indicating an inability of the bacteria to multiply in the reticulo-endothelial system (Barrow *et al.* 1999).

#### **2.4.6.1 Transmission in eggs**

The major route of transmission of *S. Pullorum* and *S. Gallinarum* is vertically via transovarial transmission; the organism being present in up to 33% of eggs laid by an infected hen (Shivaprasad 2003). Bacteria can localise in the ovules before ovulation, or can contaminate the ovum following ovulation, with the former mode considered to be the more important. A carrier state exists, with infected birds capable of infecting the next generation through vertical transmission, and other birds through faecal shedding of organisms (Shivaprasad 2003).

#### **2.4.6.2 Conclusion**

As ducks are relatively resistant to infection with *S. Pullorum* and *S. Gallinarum* and are not reservoirs of infection, it is very unlikely that these agents would be present in the breeding flocks of countries supplying hatching duck eggs to Australia. Therefore, it is recommended that the requirement for serological testing of sentinel chickens for *S. Pullorum* and *S. Gallinarum* in PAQ be removed.

Culture for other *Salmonella* spp. is currently conducted in the first 10 days post-hatch, and would therefore detect these agents without the need for specific serological testing. Routine bacteriological culture would be unaffected by the recommended change in requirements.

## **2.5 Assessment of non-OIE listed avian disease agents**

### **2.5.1 *Salmonella* Enteritidis and *Salmonella* Typhimurium DT 104**

*S. Enteritidis* and *S. Typhimurium* are typically non-host-specific bacterial pathogens, principally of concern as a major cause of food-borne salmonellosis in humans. In poultry, strains of these two *Salmonella* serovars cause systemic infection, leading to contamination of meat and eggs. *S. Enteritidis* and *S. Typhimurium* seldom cause clinical disease, except in susceptible young birds (Gast 2003). The serovars are distributed virtually worldwide in a range of species (Barrow 2000) including ducks, but significant subtypes and particular strains of concern are not endemic in Australia.

*S. Enteritidis* phage type 4 (PT 4), phage type 8 (PT 8) and phage type 13a (PT 13a) are generally recognised as the most important of the 50 or so phage types of *S. Enteritidis*. An *S. Enteritidis* outbreak involving a commercial meat-chicken company was investigated in Queensland in 2005. Control measures, including culling, stringent disinfection procedures and on-going monitoring, were put in place by Queensland State Government authorities (K Bell, Safe Food Queensland, Australia, *pers. comm.* May 2006).

More than 270 phage types of *S. Typhimurium* are recognised, of which definitive phage type 104 (DT104) is one of the most important. (Joint Expert Technical Advisory Committee on Antibiotic Resistance 1999)(1999) found that multi-drug resistant strains of *S. Typhimurium* DT 104 had not been isolated from poultry flocks in Australia.

*S. Enteritidis* PT 4 and *S. Typhimurium* DT 104 have been isolated from cases of human Salmonellosis in Australia, but are usually associated with overseas travel. There has been at least one outbreak of multi-drug resistant *S. Typhimurium* DT 104 from imported contaminated food (Promed Mail 2001). Introduction of these pathogens would have a significant impact on the Australian poultry industry through their effect on public health, animal health and trade (Crerar *et al.* 1999).

Salmonellosis due to *S. Enteritidis* or *S. Typhimurium* infection is no longer an OIE listed disease.

Transmission of salmonellae is generally via the faecal-oral route, but may also occur by the respiratory or conjunctival routes, transovarially or via the cloaca (Barrow 2000). The organisms are shed in relatively large numbers in the faeces. In the hatchery, chicks may be infected through the ingestion of contaminated fluff, shell and dust, usually leading to cross contamination of other birds (Barrow 2000). Ducks can become infected and spread the infection horizontally (Fulton *et al.* 2002).

#### **2.5.1.1 Transmission in eggs**

Intact shell eggs have been implicated as the major vehicle of transmission of *S. Enteritidis* (Cox 1995). *S. Enteritidis* has been isolated from soiled duck eggs (Baker *et al.* 1985). Although experimental studies have shown that both *S. Enteritidis* and *S. Typhimurium* are able to colonise the tissues of the reproductive tract and forming eggs at equivalent rates (Keller *et al.* 1997), transovarian contamination of commercially produced eggs with *S. Typhimurium* is rare (Keller *et al.* 1997).

The numbers of salmonellae in the contents of freshly laid eggs are usually low (<10 cfu/ml) but may be as high as 100 cfu/ml (Humphrey *et al.* 1991). The principle sites of contamination within eggs are albumen (80% positive eggs) or outside of the yolk membrane (13% positive) (Gast and Beard 1990; Humphrey *et al.* 1991)

The load of bacteria present in eggs laid by naturally infected ducks has not been recorded. Therefore it is difficult to determine if a sufficient dose of bacteria would be present in or on an infected or contaminated duck egg.

#### **2.5.1.2 Conclusion**

The 2006 import requirements state that shed litter should be cultured, eggs disinfected both pre- and post-arrival and serology for *S. Enteritidis* is to be performed on the source flock less than 21 days before egg collection. The 2006 import requirements also stipulate that samples of hatchery waste, faeces, all pipped embryos, healthy culls and all ducklings that die within

10 days of hatching are cultured to detect salmonellae. Biosecurity Australia proposes testing of samples less likely to yield salmonella, that is, faeces and healthy culls be no longer required. Sampling from hatchery waste (e.g. fluff, hatcher basket-liners and delivery-box liners), pipped embryos and duckling that die within 10 days after hatching is generally recommended and can provide sufficient assurance that salmonellae will be detected if present.

Culture for salmonella organisms is conducted in the first 10 days post-hatch, and will therefore be unaffected by the recommended change to shorten the PAQ period. Sentinel chickens infected soon after hatching develop detectable antibodies at 20-40 days of age (Shivaprasad 2003). Hence, the 2006 requirement for serological testing of sentinel chickens for *S. Enteritidis* at nine weeks post-hatch provides an additional safeguard that would not be diminished if testing were reduced to six weeks post-hatch.

## **2.5.2 Goose parvovirus and Muscovy duck parvovirus**

Goose parvovirus (GPV; previously known as goose virus hepatitis and Derzsy's disease) is a highly contagious disease of geese and young Muscovy ducks (*Cairina moschata*). GPV has been reported in Europe, the former Soviet Union, Israel, China, Taiwan, Japan and Vietnam (Gough 2003). It is not present in Australia.

GPV spreads rapidly within hatcheries from congenitally infected goslings, causing early and severe mortality (Gough 2003). The severity of disease is age-related, with mortality approaching 100% in birds infected under one week of age. Goslings over the age of four weeks may show no clinical signs. Passively acquired yolk antibodies to GPV are eliminated from hatchlings from three weeks of age (Kisary 1977).

A similar but distinct parvovirus, Muscovy duck parvovirus (MDPV), causes an acute, systemic disease of Muscovy ducklings. When first isolated in France in 1989, MDPV was considered to be a variant of GPV but it is now considered to be a distinct species.

MDPV has been described in the USA (Woolcock *et al.* 2000), Thailand (Sirivan *et al.* 1998), Germany and Malaysia (Woolcock *et al.* 2000). Japan and Taiwan have also reported MDPV disease but the diagnosis was not confirmed (Woolcock *et al.* 2000). The disease is most severe in young ducklings less than five weeks of age, but older birds are also susceptible to infection (Barnes 1997). In young ducklings, morbidity of 30% to 80% and mortality of 10% to 50% may occur (Woolcock *et al.* 2000).

Diagnosis of GPV or MDPV infection can be made by virus isolation, PCR and serological testing. Serological tests do not distinguish between GPV and MDPV (Woolcock *et al.* 2000).

### **2.5.2.1 Transmission in eggs**

GPV is vertically transmitted, resulting in congenital infections of goslings within the hatchery. Suspected vertical transmission of MDPV has also been reported (Woolcock *et al.* 2000). Adults may be infected during lay or there may be reactivation of latent infection (Barnes 1997).

### **2.5.2.2 Conclusion**

The 2006 import requirements state that there is to be no evidence of illness in the source flock from 90 days prior to egg collection and that the source flock is negative on serological

testing before egg collection. As GPV and MDPV are egg-transmitted, and infection is likely to spread rapidly within the hatchery, it is likely these diseases would be detected shortly after hatching. Also, the requirements stipulate investigation of any mortality within the PAQ flock.

Serological testing of the PAQ flock (Muscovy ducks only) at nine weeks of age for GPV is currently required. It is assumed an immune response in ducklings following exposure to virus post-hatch would occur within five weeks. The recommended serological testing at six weeks would be expected to detect any antibody response if virus was present. A negative test result at six weeks of age, rather than nine weeks provides an equivalent assurance that GPV is not present in the birds.

### **2.5.3 Reovirus of Muscovy ducks**

Reovirus infection of Muscovy ducks has been described in South Africa, France, Israel and China. Chinese isolates have also been shown to infect mule ducks, which are Muscovy-common duck hybrids (Huang *et al.* 2004). Reovirus of Muscovy ducks is not present in Australia.

Reovirus infection causes an acute disease with high morbidity and up to 10% mortality in two to four week old Muscovy ducklings (Woolcock 2003). The disease first appears at 10 days of age and persists in the affected flock until six weeks of age (Malkinson 1981; Jones 2003). Infected ducklings show characteristic lesions of the liver and spleen (Malkinson 1981) and produce neutralising antibody.

The virus may persist in breeding stock for long periods and Muscovy ducklings may be protected by maternal antibody (Malkinson 1981). Maternally derived reovirus antibodies can be detected in chickens up to three weeks of age (van der Heide 1977).

#### **2.5.3.1 Transmission in eggs**

Reoviruses can be transmitted vertically through the egg (Malkinson 1981) and therefore, infection is likely to spread rapidly within a hatchery and would be detected shortly after hatching.

#### **2.5.3.2 Conclusion**

The 2006 import requirements state that the source flock is to show no evidence of illness for 90 days prior to egg collection and to test negative on serology for reovirus of Muscovy ducks. Although post-hatch testing is not required, any infection would be detected shortly after hatching. The 2006 import requirements will not be affected by the recommended change to the PAQ period.

### 3 Summary of risk management conclusions

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The following changes (see Appendix 5.1) are recommended to Australia's import requirements for hatching duck eggs from approved countries:

1. The PAQ be shortened from 12 to 9 weeks post-hatch. Biosecurity Australia considers that this would not increase the risk of introducing exotic pathogens of quarantine concern into Australia, provided all testing of parent flocks, hatchlings and sentinel chickens is carried out and test results are received and notified in a timely manner.
2. The vaccination of donor flocks against duck hepatitis virus type I should be permitted. Details of the vaccination should be certified and there should be no requirement to test vaccinated donor flocks for DHV 1. PAQ testing should remain unchanged from the 2006 import requirements.
3. The vaccination of donor flocks against Newcastle disease virus continues to be permitted but restricted to the use of inactivated vaccines.
4. Reference to *Salmonella Pullorum* and *Salmonella Gallinarum* and the requirements to test for these agents be removed from the import requirements as ducks are relatively resistant to infection and are not known reservoirs of the agent. The post-arrival bacteriological culture for *Salmonella* spp. of pipped<sup>7</sup> embryos, hatchery waste and ducklings that die in PAQ will continue to be carried out.
5. Reference to *Salmonella Arizona* and requirements to test specifically for this agent be removed as this disease agent is present in Australia and is not considered to have an adverse impact.
6. Reference to *Riemerella anatipestifer* be removed as this agent is not egg-transmitted and is present in Australia.
7. Testing for *Salmonella Enteritidis* be revised, removing the requirement to test culled ducklings and faeces post-arrival. All other testing requirements remain; that is, pre-export testing of the source flock and post-arrival serology and bacteriological culture for *Salmonella* spp. of pipped embryos, hatchery waste and ducklings that die in PAQ will continue to be carried out.
8. Testing in PAQ for multi-drug resistant *Salmonella Typhimurium* DT104 be introduced. This will be undertaken as part of the post-arrival bacteriological culture for *Salmonella* spp. of hatchery waste, pipped embryos and duckling that die in PAQ.

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<sup>7</sup> Fully grown duck hatchlings that penetrate, but fail to hatch out of, the egg.

## 4 Reference List

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## 5 Appendices

### 5.1 Recommended quarantine requirements for the importation of hatching duck eggs from approved countries

#### REQUIREMENTS FOR THE IMPORTATION OF HATCHING DUCK EGGS FROM APPROVED COUNTRIES

##### 1. DOCUMENTATION

- a. Prior permission in writing to import fertile duck eggs must be obtained from the Australian Quarantine and Inspection Service (AQIS). The completed application should be posted or faxed to AQIS – Live Animal Imports in Canberra.

AQIS – Live Animal Imports GPO Box 858 Canberra ACT 2601 Australia	Fax +61 (2) 6272 3110 Phone +61 (2) 6272 4454
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- b. All consignments must be accompanied by a 'Permit to Import' and the appropriate Certificates which must not be modified without the written permission of AQIS. These documents must be provided to the AQIS Officer at the port of entry.
- c. Certification and post-arrival quarantine (PAQ) requirements vary, depending on the disease status of the country of origin, and vaccination status of the source flock.

##### 2. ELIGIBILITY

- a. Approved countries

Importation is only permitted from countries approved by AQIS. To be considered for approval, countries need to demonstrate an effective veterinary service, have in place appropriate surveillance programs for avian diseases and practise a policy of active eradication by stamping out outbreaks of virulent Newcastle disease and notifiable avian influenza.

- b. Highly pathogenic notifiable avian influenza

Importation is only permitted from countries officially free of highly pathogenic notifiable avian influenza in poultry, as defined in the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code.

c. Outbreaks of Newcastle disease and notifiable avian influenza

Definition of *Occurrence of Newcastle disease or notifiable avian influenza*:

For the purposes of this protocol, reference to a time interval after the occurrence of Newcastle disease or notifiable avian influenza is to be interpreted as the time interval after the last case of the disease has been reported *and* following the completion of a stamping out policy and disinfection procedures. Thus, the occurrence of a disease includes the stamping out and disinfection procedures.

**3. REQUIREMENTS RELATING TO THE SOURCE FLOCK**

- a. The source flock must not have been vaccinated against avian influenza.
- b. Vaccination of the source flock against Newcastle disease using inactivated vaccines is permitted, but must not have been conducted within 10 weeks of the date of commencement of pre-collection testing. The source flock, if vaccinated against Newcastle disease, will require additional testing to demonstrate no significant rise in antibody titres. Where any bird in the source flock is vaccinated against Newcastle disease, the entire flock shall be considered to have been vaccinated and requirements for vaccinated source flocks apply.
- c. Vaccination of the source flock against duck hepatitis virus type I is permitted.
- d. The source flock must not have been vaccinated against duck virus enteritis.
- e. The eggs must be laid by a source flock with a maximum age range of 12 weeks, the youngest bird being not less than 40 weeks old when eggs are collected, and which has been a closed flock from the onset of sexual maturity.
- f. The source flock must be housed in secure rodent-proof and bird-proof buildings and located 400 metres from all poultry unless these are shown by testing to be of a health status equal to the source flock.
- g. The source flock will be tested for specified diseases:
  - i. Pre-egg collection – not more than 21 days before the first day of collection of eggs.
  - ii. Post-egg collection – between 14 and 21 days after the last day of collection of eggs.
- h. The source flock may be exempted from testing for specified diseases where AQIS is satisfied that an official flock health monitoring program provides sufficient assurance of freedom from disease.
- i. The Declaration by the owner or manager of the source flock and the First Veterinary Certificate (requirements specified in Appendix 5.2), detailing the results of the pre-egg collection testing of the source flock will accompany the consignment of eggs.
- i. The Second Veterinary Certificate (requirements specified in Appendix 5.3), detailing the results of the post-egg collection testing of the source flock, will be supplied as soon

as possible after the post-egg collection observation period and the test results are available.

#### **4. EGG COLLECTION AND TRANSPORT**

- a. The eggs must be collected, indelibly marked and dispatched under the supervision of a Official Veterinarian of the country of export. The eggs must undergo fumigation or disinfection and then must be packed and sealed in airtight, leak-proof containers for transport to Australia.
- b. The eggs must be packed in such a way that there will be no leakage in the event of the eggs breaking during transport.
- c. The eggs must be consigned to Australia by air, using a route approved by AQIS. They may be accompanied in transit by other eggs or birds only with the prior approval of AQIS. Any transshipment requires the prior approval of AQIS.
- d. In the event of a consignment arriving in Australia in an unsealed container, or in a container the seal of which has been broken, or with inadequate certification, the consignment may not be permitted entry into Australia.

#### **5. QUARANTINE**

- a. The imported eggs will be hatched in a Quarantine Approved Premise (QAP). The quarantine flock of ducklings which are hatched from these eggs will remain in quarantine for a period of nine weeks, and will only be released subject to satisfactory results of a program of testing during quarantine as prescribed by AQIS. The PAQ testing requirements are specified in Appendix 5.4.
- b. AQIS may approve a QAP based on criteria set out in AQIS's guidelines on the location and construction of such a facility. The use of the facility for the quarantine of hatching eggs must be subject to quality assurance based systems approved by AQIS; approval will be dependent on the importer and the operator of the QAP agreeing to comply with policies, procedures and specifications set out in an Approved Quarantine Directive Manual.
- c. The facility must be so constructed as to ensure that the eggs during incubation and hatching, and the ducklings after hatching, are contained within a ventilation system that is HEPA filtered until the successful completion and reporting of the results of all post-egg collection testing of the source flock.
- d. A sentinel flock of chickens must be hatched and reared concurrently with the quarantine flock in PAQ in a ratio of 1 sentinel to 50 quarantine birds. In the case where the 1:50 ratio results in the number of sentinel chickens being less than 100, a minimum of 100 sentinel chickens must be reared concurrently with the quarantine flock.
- e. In these requirements, the word 'disease' means a disease or disease agent as listed below:
  - Highly pathogenic notifiable avian influenza
  - Low pathogenicity notifiable avian influenza

Newcastle disease  
Duck hepatitis virus type I  
Duck hepatitis virus type II  
Duck hepatitis virus type III  
Duck virus enteritis  
Infectious bursal disease virus (very virulent and exotic variant strains)  
*Salmonella* Enteritidis  
*Salmonella* Typhimurium DT104  
Goose and Muscovy duck parvoviruses (Muscovies only)  
Reovirus infection of Muscovy ducks (Muscovies only).

- f. Approved tests described in Appendices 5.1, 5.2, 5.3, 5.4 and 5.5 are listed in Appendix 5.6. In the case of testing for avian influenza virus and Newcastle disease virus, the sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was 5% disease prevalence in the source flock. For the other diseases listed, the sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was 0.5% disease prevalence in the source flock (see Appendix 5.7).

## **6. IMPORTER'S / AGENT'S RESPONSIBILITIES**

- a. The importer or the agent coordinating the importation must be Australian based and must nominate a person who will be accessible to AQIS officers if any problems or emergencies arise.
- b. If, during the process of quarantine, it is found that the pre-export testing or certification requirements have not been fully met, the consignment may be re-exported or destroyed.
- c. The agent and the aircraft operator are responsible for the safe transportation of the eggs.
- d. All costs associated with the testing, transport, quarantine and veterinary supervision during the importation program must be met by the importer/agent.
- e. If any eggs or birds are destroyed during any period of control, compensation will not be paid by the Government.
- f. The diseases included in Section 5e of these requirements are of quarantine concern. It is the prerogative of the importer to arrange for any other health certification or testing of the fertile duck eggs for export or the birds hatched from the imported eggs.

## **7. ACTION TO BE TAKEN FOLLOWING THE DETECTION OF A PATHOGEN IN BIRDS IN QUARANTINE IN AUSTRALIA**

If an investigation or specified test indicates the presence of a pathogen (as defined in Section 5e of these requirements) in the quarantine flock (including sentinel birds), AQIS must be notified and the flock must remain in quarantine. At the discretion of AQIS and in consultation with the laboratory carrying out the investigations or tests and, where necessary, other relevant authorities, further investigations and additional testing

may be carried out to ascertain the cause of the positive result. The quarantine flock may be destroyed if it is confirmed that it is infected with any of the diseases listed in Section 5e or, at the discretion of AQIS, with any other pathogen. Any decision by AQIS must be made in consultation with the Australian states, industry and scientific organisations.

## **8. REVIEW**

The requirements of importation may be reviewed if there are any changes in the animal health situation and/or the import policy of the exporting country or at any time at the discretion of the Director of Animal and Plant Quarantine.

## 5.2 Requirements for the first veterinary certificate

### First Veterinary Certificate Export of hatching eggs of ducks to Australia (pre-egg collection)

- This certificate is to accompany the consignment of eggs.
- This certificate is to be signed and dated both by the owner or manager of the source flock and an Official Veterinarian<sup>8</sup>.
- All pages are to be endorsed with the Official Stamp.

#### **PART A: Declaration by the owner or manager of the source flock**

- This Part is to be headed by a statement detailing the name of the owner or manager of the source flock from which the eggs to be exported to Australia are derived and a declaration as to the following:
  1. The source flock has not been vaccinated against avian influenza.
  2. Newcastle disease:
    - the source flock has not been vaccinated against Newcastle disease
    - or
    - the source flock has been vaccinated against Newcastle disease using an inactivated vaccine more than 10 weeks prior to the commencement of pre-egg collection testing.
  3. Duck hepatitis virus type I:
    - the source flock has not been vaccinated against DHV-type I
    - or
    - the source flock has been vaccinated against DHV-type I more than 10 weeks prior to the commencement of pre-egg collection testing.
  4. The source flock has not been vaccinated against duck virus enteritis.
  5. The vaccination history of the source flock detailing:
    - the disease agents vaccinated against
    - the dates of vaccination
    - the vaccines used.
  6. The eggs have been laid by a source flock that has a maximum age range of 12 weeks and that has been a closed flock since the onset of sexual maturity.

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<sup>8</sup> As defined in the OIE Animal Terrestrial Animal Health Code 2008

## **PART B: Disease status of the country of origin**

- This Part is to be headed by a statement showing the exporting country and the name of the Official Veterinarian and a declaration as to the following:
1. The exporting country is free from highly pathogenic notifiable avian influenza (HPNAI) as defined by the OIE Terrestrial Animal Health Code.
  2. Low pathogenicity notifiable avian influenza (LPNAI)
    - The country/zone of export is currently free from LPNAI as defined by the OIE Terrestrial Animal Health Code
    - or
    - The country/zone of export is not free from LPNAI, but stamping out and disinfection procedures were completed greater than 21 days prior to the start of collection of eggs for this consignment; and no case of LPNAI has been reported in the country or zone during the egg-collection period
    - or
    - The country/zone of export is not free from LPNAI but the source flock is located on premises which are more than 40 kilometres from the nearest case. LPNAI has not occurred on any premises associated with, including common ownership, the source flock within the six months previous to the start of collection of eggs for this consignment.
  3. Newcastle disease
    - The country/zone of export is currently free from Newcastle disease as defined by the OIE Terrestrial Animal Health Code
    - or
    - The country/zone of export is not free from Newcastle disease but stamping out and disinfection procedures were completed greater than 21 days prior to the start of collection of eggs for this consignment; and no case of Newcastle disease has been reported in the country or zone during the egg-collection period
    - or
    - The country/zone of export is not free from Newcastle disease but the source flock is located on premises which are more than 40 kilometres from the nearest case. Newcastle disease has not occurred on any premises associated with, including common ownership, the source flock within the six months previous to the start of collection of eggs for this consignment.
  4. The country/zone of export has been free of disease caused by the following agents\* for a period of at least six months:
    - Duck hepatitis virus type II
    - Duck hepatitis virus type III
    - Duck virus enteritis
    - *Salmonella* Enteritidis
    - *Salmonella* Typhimurium DT104
    - Goose and Muscovy duck parvovirus (Muscovies only)
    - Reovirus infection of Muscovy ducks (Muscovies only)

\* Delete those disease agents that are not applicable

## **PART C: Flock status and disease testing**

- This part is to be headed by a statement showing the exporting country and the name of the Official Veterinarian and a declaration as to the following:
1. The source flock, from which the eggs to be exported to Australia were derived, has been under my supervision for the previous 90 days.
  2. A biosecurity program has been in place during the period under my supervision. This included the use of dedicated staff for the source flock, movement control and disinfection of vehicles not dedicated to the source flock facility, such as those for waste removal, feed delivery and service personnel vehicles. After due enquiry I am satisfied that there has been no epidemiological contact between the source flock and any premises on which clinical Newcastle disease, avian influenza, duck viral hepatitis or duck viral enteritis has occurred within six months prior to the commencement of egg collection until despatch of this consignment.
  3. All water supplied to the source flock is secure against contamination by wild birds.
  4. The source flock is housed in secure rodent-proof and bird-proof buildings and is isolated by more than 400 metres from all poultry which have not been shown by testing to be of a health status equal to the source flock. Details of poultry within 400 metres of the source flock are attached.
  5. The source flock has been free from clinical signs of the following disease agents for the 90 day period prior to collection of the eggs and during egg collection and has not come into contact with any birds showing evidence of these disease agents:
    - Highly pathogenic avian notifiable influenza virus
    - Low pathogenicity notifiable avian influenza virus
    - Newcastle disease virus
    - Infectious bursal disease virus
    - Duck hepatitis virus type I
    - Duck hepatitis virus type II
    - Duck hepatitis virus type III
    - Duck virus enteritis
    - *Salmonella* Enteritidis
    - *Salmonella* Typhimurium DT104
    - Goose and Muscovy duck parvovirus (Muscovies only)
    - Reovirus infection of Muscovy ducks (Muscovies only)
  6. **Pre-egg collection testing – avian influenza**
    - a. Serology

Within 21 days before the first day of collection of eggs for export to Australia, a sample of the parent flock was tested serologically negative for antibody to avian influenza. The sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was 5% disease prevalence in the source flock.
    - b. Virus isolation

Within 21 days before the first day of collection of eggs cloacal swabs were collected from a sample of birds in the source flock. Each sample and each bird was identified so that a second sample could have been collected at a later

stage from any specified bird. The sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was 5% disease prevalence in the source flock. Cloacal swabs from groups of no more than five birds were pooled and tested for freedom from haemagglutinating agents by direct inoculation of the allantoic cavity of 9 to 11 day-old chick embryos with cloacal swabs. No avian influenza virus was isolated.

**7. Pre-egg collection testing – Newcastle disease**

a. Serology

- i. *Unvaccinated Flock*: Within 21 days before the first day of collection of eggs for export, a sample of the parent flock was tested negative for antibody to Newcastle disease. The sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was a 5% disease prevalence in the source flock.
- ii. *Vaccinated Flock*: Within 21 days before the first day of collection of eggs, a random sample of 100 individually identified birds in the source flock was tested for Newcastle disease, with individual titres recorded for each bird sampled. A list of titres is attached to this certificate. After sampling, the 100 individually identified birds were returned randomly throughout the source flock. The test is scheduled to be repeated on the same birds not less than 14 days after the collection of the last egg for this consignment. The results of the second test are recorded and attached to the second veterinary certificate.

b. Virus isolation

Within 21 days before the first day of collection of eggs cloacal swabs were collected from a sample of birds in the source flock. Each sample and each bird was identified so that a second sample could have been collected at a later stage from any specified bird. The sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was a 5% disease prevalence in the source flock. Cloacal swabs from groups of no more than five birds were pooled and tested for freedom from haemagglutinating agents by direct inoculation of the allantoic cavity of 9 to 11 day-old chick embryos with cloacal swabs. No Newcastle disease virus was isolated.

**8. Pre-egg collection testing—serology**

Within 21 days before the first day of collection of eggs for export to Australia, a sample, of sufficient size to give a 99% confidence of detecting the disease if there was 0.5% disease prevalence in the flock, of the source flock was tested serologically for antibody to the following disease agents\* with negative results:

- Duck hepatitis virus type I (if unvaccinated)
- Duck hepatitis virus type II
- Duck hepatitis virus type III
- Duck virus enteritis
- *Salmonella* Enteritidis
- Goose parvovirus (Muscovies only)
- Reovirus infection of Muscovy ducks (Muscovies only)

\* Delete diseases for which certification of country freedom has been provided in Part B.

## 5.3 Requirements for the second veterinary certificate

### Second Veterinary Certificate Export of hatching eggs of ducks to Australia (post-egg collection)

- This certificate is to be supplied as soon as possible after the post-egg collection observation period and the test results are available.
- All pages are to be endorsed with the Official Stamp.
- The declaration is to be signed and dated by the Official Veterinarian.
- It is to be headed by a statement showing the exporting country, the name of the Official Veterinarian and a declaration as to the following:

#### **PART A: Disease status of the country of origin**

1. The country of export remains officially free of highly pathogenic notifiable avian influenza (HPNAI).
2. Low pathogenicity notifiable avian influenza (LPNAI) has not been reported within 40 kilometres of the location of the source flock during the post-egg collection period.
3. Newcastle disease has not been reported within 40 kilometres of the location of the source flock during the post-egg collection period.
4. After due enquiry, I am satisfied that the source flock has remained closed. Clinical evidence of disease has been investigated and the results indicate diseases caused by the following agents have not occurred during the period since collection of eggs:
  - Highly pathogenic notifiable avian influenza (HPNAI) virus
  - Low pathogenicity notifiable avian influenza (LPNAI) virus
  - Newcastle disease virus
  - Duck hepatitis virus type I
  - Duck hepatitis virus type II
  - Duck hepatitis virus type III
  - Duck virus enteritis
  - *Salmonella* Enteritidis
  - *Salmonella* Typhimurium DT104
  - Goose and Muscovy duck parvovirus (Muscovies only)
  - Reovirus infection of Muscovy ducks (Muscovies only)

## **PART B: Flock status and disease testing**

1. The source flock, from which the eggs were derived, has been under my supervision for the 21 days since the eggs exported to Australia were collected.

2. **Post-egg collection testing – avian influenza**

Between 14 and 21 days after the last day of collection of eggs, cloacal swabs were collected from a sample of birds in the source flock. The sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was 5% disease prevalence in the flock (see Appendix 5.7). Each sample and each bird was identified so that a second sample could have been collected at a later stage from any specified bird. Cloacal swabs from groups of no more than five birds were pooled and tested for freedom from haemagglutinating agents by direct inoculation of the allantoic cavity of 9 to 11 day-old chick embryos. No avian influenza virus was isolated.

3. **Post-egg collection testing – Newcastle disease**

- a. Serology

*Vaccinated Flock:* Between 14 and 21 days after the last day of egg collection for this consignment the same 100 birds individually identified in the pre-egg collection testing were again tested. The results of this second test were recorded and are attached to this veterinary certificate.

- b. Virus isolation

Between 14 and 21 days after the last day of collection of eggs, cloacal swabs were collected from a sample of birds in the source flock. The sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was 5% disease prevalence in the flock (see Appendix 5.7). Each sample and each bird was identified so that a second sample could have been collected at a later stage from any specified bird. Cloacal swabs from groups of no more than five birds were pooled and tested for freedom from haemagglutinating agents by direct inoculation of the allantoic cavity of 9 to 11 day-old chick embryos. No Newcastle disease virus was isolated.

4. All tests were carried out in a government approved laboratory or a laboratory approved by the government of the exporting country for this specific purpose and approved by AQIS. The tests were OIE approved tests or tests approved by AQIS (see Appendix 5.6).
5. Any clinical disease in the source flock or drop in quantity, quality, or fertility/hatchability of the eggs produced by the source flock has been investigated and the laboratory reports are attached.
6. Results of post-egg collection testing are attached.

## 5.4 Requirements for the certificate from the veterinary officer supervising the post-arrival quarantine approved facility

### Certificate from the Veterinary Officer supervising the post-arrival quarantine (PAQ) approved facility

- This certificate to be supplied before the flock is released from PAQ.
- It should show the following details of the imported Consignment
  - Consignor
  - Date of arrival
  - Identification of consignment.
- All pages are to be endorsed with the Official Stamp.
- The certificate is to be signed and dated by the veterinary officer supervising the approved PAQ facility.
- The certificate will contain a declaration as to the following:
  1. The consignment of eggs described above was carried directly from the aircraft to the egg hatchery. After arrival, the eggs were stacked on plastic egg flats and were either:
    - fumigated with formaldehyde gas
    - or
    - disinfected using Virkon® or Oxy RTU®.
  2. All packing materials consigned with the imported eggs were either:
    - autoclaved prior to disposal
    - or
    - incinerated
    - or
    - held within the PAQ facility until the consignment is released.
  3. The eggs were then incubated to hatch the quarantine flock.
  4. A quantity of eggs from Australian Specific Pathogen Free (SPF) flocks were treated similarly and incubated to hatch the sentinel flock. The ratio of Australian SPF chicks to imported ducklings at the day-old stage was at least 1:50 with a minimum of 100 sentinel chickens reared concurrently with the quarantine flock.
  5. To the best of my knowledge, the eggs and imported hatched and sentinel birds in the QAP were observed daily for evidence of disease. Where abnormalities were observed and reported to me, a full investigation was carried out and a report is attached.
  6. Virus testing: cloacal swabs were collected from a sample of birds in the quarantine flock at six weeks of age. The sample tested was of a sufficient size to give a 99% confidence of detecting the diseases tested if there was 5% disease prevalence in the quarantine flock. Each sample and each bird was identified so that a second sample could have been collected later

from any specified bird. Cloacal swabs from groups of no more than five birds were pooled and tested for freedom from haemagglutinating agents by direct inoculation of the allantoic cavity of 9 to 11 day-old chick embryos. Any agents isolated were specifically identified and avian influenza virus and Newcastle disease virus were absent.

7. Serology: a sample of the quarantine flock and all sentinel birds were bled at six weeks of age. The sample tested was of a sufficient size to give a 99% confidence of detecting the diseases tested if there was 0.5% disease prevalence in the quarantine flock. Sufficient blood was collected from each bird sampled for the performance of the required tests. Each sample and each bird was identified so that a second sample could have been collected later from any specified bird.

8. Each serum sample collected was tested for antibodies to the following pathogens:

Quarantine birds:

- Duck virus hepatitis virus type I
- Duck enteritis virus
- Goose and Muscovy duck parvovirus (Muscovies only)

Sentinel chickens:

- Avian influenza virus
- Newcastle disease virus
- *Salmonella* Enteritidis
- Infectious bursal disease virus

Where there were positive or suspicious reactors for *Salmonella* Enteritidis, all reactors were killed and their organs cultured, and the results of the tests are attached.

9. The absence of *Salmonella* Enteritidis and *Salmonella* Typhimurium DT104 was determined by microbial monitoring techniques. Isolations of *S. Pullorum* or *S. Gallinarum* have been notified to AQIS.

10. The program was audited according to the requirements set out in the Approved Quarantine Manual. As far as could be ascertained, during the quarantine period, all appropriate security measures with respect to the egg hatchery, to staff associated with the hatchery and to all materials entering or leaving the hatchery were taken. As far as could be ascertained, at no stage during the quarantine period was there a breakdown in security at the QAP.

11. Attached are the reports from the Approved Government Laboratory which identify the samples tested with the birds in the quarantine and sentinel flocks, establish the validity of all tests and state the results of all tests.

12. Attached are the two certificates (with enclosures) which were received from the Official Veterinarian of the Country of Export which refer to this consignment.

13. After consideration of all relevant information, I certify that the progeny of this consignment are (or are not) qualified to be released from quarantine.

## 5.5 Summary of testing requirements

Type of test	Timing			
	PEQ		PAQ	
	< 21 days pre-egg collection.	14-21 days post-egg collection	0-10 days post-hatch	6 weeks post-hatch
<i>Serology (source flock)</i>	Avian influenza (AI), Newcastle disease (ND)	ND (if vaccinated)		
<i>Serology (source flock). If not certified country-free</i>	Duck hepatitis virus (DHV) type I (if unvaccinated), DHV type II, DHV type III, Duck virus enteritis (DVE), <i>Salmonella</i> Enteritidis, Goose parvovirus*, Reovirus of Muscovy ducks *			
<i>Agent isolation (source flock)</i>	AI, ND	AI, ND		
<i>Serology (sentinel chickens)</i>				AI, ND, <i>Salmonella</i> Enteritidis, Infectious bursal disease virus
<i>Serology (PAQ flock)</i>				DHV type I, DVE, Goose parvovirus*
<i>Agent isolation (hatchery)</i>			<i>Salmonellae</i> in hatchery waste, pipped embryos, mortalities	
<i>Agent isolation (PAQ flock)</i>				AI, ND

\*Muscovy ducks only

## 5.6 Approved tests for the importation of hatching duck eggs

### 1. Approved tests for the importation of fertile eggs (Domestic Ducks)

PATHOGEN	TESTS	METHOD
Avian influenza virus	ELISA	AAHL
	AGID	OIE, SCAHLS
Newcastle disease virus	HIT	AAHL, OIE, SCAHLS
Infectious bursal disease virus	AGID, VN	OIE
<i>Salmonella</i> Enteritidis	ELISA	AAHL, OIE
	RSAT	AAHL, OIE
Goose parvovirus	WBAT	SCAHL
	SN, AGID, ELISA	
Duck enteritis virus	SN	OIE
Duck hepatitis virus type I	SN	OIE
<i>Salmonella</i> spp.	microbiological	AAHL

HIT            haemagglutination inhibition test  
 ELISA        enzyme-linked immunosorbent assay  
 RSAT        rapid serum agglutination test  
 AGID        agar gel immunodiffusion test  
 S/VN        serum/virus neutralisation test  
 WBAT        whole blood tube agglutination test

OIE            World Organisation for Animal Health  
 AAHL        Australian Animal Health Laboratory  
 SCAHLS     Subcommittee on Animal Health Laboratory Standards, Australia

### 2. Bacteriological culture of *Salmonella* spp.

#### i. Pipped embryos:

All pipped embryos are to be sampled. The livers and alimentary tracts are to be removed aseptically and placed in separate containers, either individually or in pools of up to 20. Containers must be labelled so that sample pairs can be identified.

#### ii. Mortalities:

All birds which die within 10 days after hatching are to be sampled. The liver and caecum are to be removed aseptically from each bird, keeping the two separate. Specimens are preferably submitted individually or if large numbers are involved may be pooled into groups of up to

five. Containers must be labelled to allow sample pairs to be identified. Mortality rates of <5% can be expected (<50 pools of 5).

iii. Hatchery waste:

Representative samples of fluff, shell debris and membranes are to be collected from each hatching tray and pooled. Specimens from each incubator are to be kept separate.

Approximately 200 ml of material may be collected from each tray, 2000 ml per incubator (a subsample of up to 200g per incubator should be cultured).

## 5.7 Sample size for 99% confidence of detecting 0.5% and 5% prevalence of disease

Population Size	Sample Size to detect 0.5% prevalence	Sample Size to detect 5% prevalence
10	10	10
20	20	20
30	30	30
40	40	36
50	50	42
60	60	47
70	70	51
80	80	54
90	90	57
100	100	59
120	120	63
140	140	67
160	160	69
180	179	71
200	198	73
250	244	76
300	286	78
350	325	80
400	360	81
450	392	82
500	421	83
600	470	84
700	512	85
800	546	85
900	576	86
1000	601	86
1200	642	87
1400	674	87
1600	699	88
1800	720	88
2000	737	88
3000	792	89
4000	821	89
5000	840	89
6000	852	90
7000	861	90
8000	868	90
9000	874	90
10000	878	90
$\infty$	919	90

