Proposal to Reduce the Post Arrival Quarantine Period for Imported Hatching Eggs

Background

In February 2003, Biosecurity Australia received a request from the Torrens Island Avian Quarantine Station Stakeholder Group, to reconsider the length of the post-arrival quarantine (PAQ) period for chicks hatched from imported eggs. The Torrens Island Avian Quarantine Station Stakeholder Group is made up of users of the quarantine station and the Australian Quarantine and Inspection Service (AQIS). Their request arose from a desire to achieve higher throughputs at the Torrens Island quarantine station in particular, in order to reduce the backlog of applications for space in the facility.

The request stated that reducing the PAQ period from 12 weeks post-hatch to 9 weeks post-hatch would allow an additional consignment to be accommodated, every 2 years.

The request also provided information suggesting that such a reduction in PAQ period could be achieved without increasing the quarantine risk to Australia. Copies of the request, and supporting submissions from Dr Peter Scott, consulting veterinarian to Hy-line Australia Pty. Ltd. and Dr Peter Groves, Veterinary Consultant of Zootechny Pty. Ltd., are attached at Appendices 1 to 3 respectively.

At the time of writing, there have been a total of 26 successful imports through the Torrens Island Quarantine station. There has never been a case of an exotic pathogen being detected during PAQ.

There are currently three sets of conditions for the import of hatching eggs – one each for domestic hens, turkeys and ducks.

This paper will, for domestic hens and turkeys:

- summarise the existing quarantine requirements;
- discuss the changes required to accommodate a reduced PAQ period, and
- make recommendations for a reduction in the quarantine period for hatching eggs of domestic hens and turkeys.

Conditions for the import of hatching eggs of domestic ducks will undergo further review, before any changes in the existing Conditions are considered.

Existing policy (Domestic hen eggs)

The current “CONDITIONS FOR THE IMPORTATION FROM APPROVED COUNTRIES OF FERTILE EGGS (DOMESTIC HEN)” define the following as diseases for the purposes of the “Conditions”:

Arizona disease (*Salmonella* Arizona)

Avian influenza (AI)

Avian *Paramyxoviridae* type 2 and 3 infection (PMV-2 and PMV-3)

Fowl typhoid (*Salmonella* Gallinarum)

Infectious bursal disease (IBD)

Mycoplasma iowae infection

Newcastle disease (ND)

Ornithobacterium rhinotracheale infection

Pullorum disease (*Salmonella* Pullorum)

Runting/stunting syndrome

S*almonella* Enteritidis infection

Turkey viral rhinotracheitis (avian pneumovirus) (TRT).

A copy of the "Conditions", describing the import conditions and testing requirements in detail, is included as Appendix 4.

When the "Conditions" were developed, the PAQ period was set at 12 weeks post-hatch for a number of reasons. These included:

- to ensure that sufficient time had elapsed post-hatch for any infection in the quarantine birds to spread, either to more individuals in the quarantine flock, or to sentinel birds;
- to ensure that sufficient time had elapsed for clinical signs to be shown by the quarantine flock or sentinel flock;
- to ensure that sufficient time was available for all required testing to be undertaken, and for results of that testing to become available.

Any proposal to shorten the PAQ period must be assessed to determine whether it would adversely affect any of these objectives.

Current testing regime

In addition to the requirements for general observation of source flock health over the period before and after egg collection, the "Conditions" set out a detailed program of testing in the source and quarantine flocks designed to identify low-level infection with a number of the diseases of quarantine concern. The testing regime is summarised in Table 1 below. Note that specific testing for *Mycoplasma iowae*, runting stunting syndrome and *Ornithobacterium rhinotracheale* is not currently required. However, these diseases would be detected on investigation of morbidity or mortality (including reduced hatchability) in the source and quarantine flocks, as required in the "Conditions".

From Table 1, it can be seen that final testing of the quarantine flock and the sentinel birds is currently undertaken at 9 weeks post-hatch. It can take up to 3 weeks for results of virus isolation testing to be known, and therefore the full PAQ period has been set at 12 weeks post-hatch.

Effect of proposal to shorten the PAQ period.

In order for the PAQ period to be shortened to 9 weeks, while still allowing sufficient time for testing to be completed before release of the imported birds from quarantine, the final round of samples for testing would have to be taken at 6 weeks of age. This would require that the serological testing for IBD currently undertaken at 6 and 9 weeks be reduced to testing at 6 weeks post-hatch only. All other testing requirements would be carried out at 6 weeks, rather than 9 weeks post-hatch. The effect of this change on the risk associated with each of the diseases concerned will be assessed individually.

Table 1: Current testing requirements for hatching eggs of domestic hens

Type of test	Timing				
	PEQ		*PAQ*		
	< 21 days before egg collection.	***14 – 21 days post egg collection***	***0 – 10 days post hatch***	***6 weeks post hatch***	***9 weeks post hatch***
Serology (parent flock)	AI, ND, PMV2, PMV3, TRT, *Salmonellae*	AI, ND, TRT (if vaccinated)			
Serology (sentinels)				IBD	AI, ND, TRT, IBD *Salmonellae*
Agent isolation (wastes, litter etc)	*Salmonellae* in shed litter		*Salmonellae* in hatchery waste, dead birds, healthy culls, shed litter		
Agent isolation (quarantine birds)					AI, ND, PMV2, PMV3, IBD

Infectious Bursal Disease

Infectious bursal disease (IBD) is an acute, contagious viral infection, which causes immunosuppression in young chicks, and disease and mortality in 3- to 6-week-old chickens (Lukert and Saif 1997; van den Berg et al. 2000). The virus infects actively dividing B lymphocytes within the bursa of Fabricius, leading to immunosuppression of varying duration and severity, and causing increased susceptibility to secondary viral and bacterial infections. 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). Classical and Australian variant strains which exist in Australia can be genetically differentiated from overseas classical, variant and very virulent strains (Sapats and Ignjatovic 2000; Ignjatovic and Sapats 2002). Infectious bursal disease is an OIE list B disease.

IBD virus is distributed worldwide and occurs in all major poultry producing areas, with the possible exception of New Zealand, where an attenuated (vaccine) strain was first detected in domestic poultry in 1993 (Chai et al. 2001). Strains of the classical form that exist in Australia are genetically different from IBD viruses isolated overseas (Sapats and Ignjatovic 2000; Ignjatovic and Sapats 2002). Antigenic variant viruses have been identified in Australia (Sapats and Ignjatovic 2000) and North America (Snyder, Vakharia, and Savage 1992). However these strains are only distantly related to each other (Sapats and Ignjatovic 2000), and vaccines currently used in Australia do not protect against variant IBD virus strains from North America (Ignjatovic, Sapats, and Gould 2001). Very virulent strains are exotic to Australia, but are found in Europe, Asia and South America (van den Berg, Gonze, and Meulemans 1991; Lin et al. 1993; Di Fabio et al. 1999; Ikuta et al. 2001).

IBD virus is a highly contagious virus, which is able to persist in the environment between outbreaks. The main route of transmission is via the faecal-oral route and the virus can survive for prolonged periods in faeces and bedding (Benton, Cover, and Rosenberger 1967). Mechanical transmission of virus can occur via faecal contamination of fomites (van den Berg et al. 2000). The lesser mealworm (larva of the darkling beetle) is common in poultry houses worldwide and can serve as a reservoir for the virus (McAllister et al. 1995). IBD virus has been isolated from mosquitos and rats but there is no evidence they act as vectors (Lukert and Saif 1997).

Chickens are most susceptible to infection during active bursal development, with clinical signs being most common between 3 and 6 weeks of age. Chickens infected before 3 weeks of age, and not protected by maternal antibodies, develop immunosuppression, which can lead to secondary viral and bacterial infections and reduce the efficacy of vaccination. Clinical cases may rarely be seen in birds up to the age of 15-20 weeks (Ley et al. 1979; Okoye and Uzoukwu 1981). Although age-resistance to the development of clinical signs of IBD has been documented, little is known about age-resistance to IBD virus infection in chickens. In susceptible birds, the incubation period is as short as 2-3 days.

Maternal antibodies can protect chicks against early infections with classical strains of IBD virus, with resultant protection against the immunosuppressive effects of the virus. However, maternal antibody does not protect chicks from disease associated with very virulent strains, unless present at high levels, and may be poorly protective against some variant strains (Lukert and Saif 1997). Vaccination of parent birds with inactivated oil emulsion vaccines, following initial vaccination with a live virus, appears to afford longer-lasting passive immunity than vaccination with attenuated live vaccines (Lukert and Saif 1997).

Transmission in eggs

There is no evidence that IBD virus is transmitted vertically (Lukert and Saif 1997; van den Berg et al. 2000). While faecal contamination of the surface of the shell with virus could occur, most hens of laying age would be immune or resistant to infection with IBD virus, and would be unlikely to shed virus in the faeces. In an unpublished experimental trial, IBD virus was not isolated from the yolk, albumen or shell of eggs laid by non-vaccinated 24-week old chickens challenged with vvIBD virus, despite the presence of virus in cloacal swabs for 2-3 days after inoculation (unpublished report, Australian Animal Health Laboratory).

Conclusions

Taking the above information into account, it would appear that there is an extremely low likelihood that there will be IBD virus in imported hatching eggs, particularly as the “Conditions” require that there has been no evidence of illness in the source flock from 90 days prior to commencement of egg collection, and that the source flock has a minimum age of 35 weeks. However, there remains the very low likelihood that the external shell of imported eggs may be contaminated, and may lead to infection of hatchlings shortly after hatch. To mitigate this risk, the “Conditions” require that all hatching eggs be disinfected after collection, and again on arrival in PAQ. Currently, the “Conditions” specify the use of formalin fumigation, or an alternative approved by the Director. There is some doubt as to the efficacy of formalin fumigation against IBDV, but Virkon S is an acceptable alternative for this purpose, and also has been shown to be effective against a range of other poultry pathogens, including Newcastle disease virus, and avian influenza virus. An amendment to the “Conditions” to specify the use of Virkon S or equivalent egg disinfectant, which has been

shown to be effective against IBDV, should reduce the likelihood of shell contamination to negligible. If these factors were not sufficient to prevent infection, it is highly likely that any IBD which did infect the quarantine flock, particularly very virulent or virulent variant strains, would spread rapidly among the quarantine flock and the sentinel birds, and would be readily recognised at post mortem of affected birds.

It is therefore considered that the serological test undertaken on sentinel birds at 6 weeks post-hatch, in combination with virus isolation testing on quarantined birds, provides sufficient additional confidence that IBD is not present in the imported birds, and the shortening of the PAQ period would not significantly increase the risk associated with IBD.

Avian Influenza

Most avian influenza (AI) viruses are of low pathogenicity (LP), producing either subclinical disease or mild respiratory or reproductive disease in domestic and wild birds. Highly pathogenic (HP) AI, formerly known as fowl plague, is a highly contagious systemic disease of poultry that causes high mortality. While LPAI viruses circulate widely in wild bird populations, HPAI viruses do not have a recognised wild bird reservoir. HPAI viruses have been documented to arise from mutations in LPAI viruses, with mutations probably occurring within domestic poultry populations (Swayne and Suarez 2000). HPAI is an OIE List A disease.

There were 18 documented outbreaks of HPAI in the English language literature between 1955 and 2000 (Swayne and Suarez 2000), and, in 2003, outbreaks of H7N7 HPAI were reported in The Netherlands, Belgium and Germany (Shane 2003). In 2004, there were widespread avian influenza (H5N1 and others) outbreaks of unprecedented magnitude in Asia, involving at least 10 countries.

Outbreaks of HPAI occurred in Australia in 1976, 1985, 1992, 1995 and 1997 (Swayne and Suarez 2000). In each instance, the virus was believed to have originated from wild water birds, with direct contact between birds and poultry, or faecal contamination of a water source, being implicated in the outbreak of disease in poultry (Morgan and Kelly 1990; Animal Health Australia 1995; Selleck et al. 1997; Anonymous 1998a). In the 1985 outbreak in Victoria, chickens showed mild clinical signs (10-20% reduction in egg production and mild respiratory disease) for several weeks before a sudden increase in death rate occurred (30-80% in different sheds on the same farm), suggesting that the birds were initially infected with a mildly pathogenic virus that became highly pathogenic after establishment in poultry. The 1997 outbreak in New South Wales was attributed to faecal contamination of river water by wild birds, with aerosol and mechanical spread occurring from the first infected farm to two other farms nearby. Mortality rate in affected sheds on the first farm varied from 40-100% (Anonymous 1998a). Australia is currently considered free of HPAI.

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, Hinshaw, and Halvorson 1997). The incubation period depends on the dose of the virus, route of exposure and the species exposed, as well as ability to detect clinical signs.

Serological tests are used to demonstrate the presence of antibodies that may be detected as early as 7-10 days after infection. However, there is considerable variation in the immune response among the various avian species (Easterday, Hinshaw, and Halvorson 1997).

Transmission in eggs

Avian influenza virus can be present within, or on the surface of, eggs laid by naturally-infected hens (Easterday, Hinshaw, and Halvorson 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 HPAI in Pennsylvania (Cappucci et al. 1985). Data from that study indicated that the virus can survive for at least several days in the albumen and yolk of eggs stored at 10-18 ºC.

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

Conclusions

The "Conditions" require that there is no evidence of illness in the source flock from 90 days prior to egg collection, and that the source flock is not vaccinated against AI. However, there remains the small likelihood that the external shell of imported eggs may be contaminated, and may lead to infection of hatchlings shortly after hatch. To mitigate this risk, the "Conditions" require that all hatching eggs are fumigated after collection, and again on arrival in PAQ. This should reduce the likelihood of shell contamination to negligible. Given the highly contagious nature of AI viruses, and the short incubation period, AI would spread rapidly among the quarantine flock and sentinel birds. If present, AI would be readily diagnosed within the 6-week post-hatch testing period.

Newcastle disease

Strains of Newcastle disease (ND) virus 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, ND virus strains are broadly classified as velogenic (highly virulent), mesogenic (moderately virulent), lentogenic (mildly virulent) and avirulent (Alexander 1997).

The natural hosts of ND virus are domestic poultry, including chickens, turkeys, ducks, geese, pigeons, quail, pheasants, guinea fowl and ostriches, and many species of captive caged birds and wild birds (Alexander 2000). Susceptibility varies between species, with chickens the most likely to show clinical ND, and water birds the least likely to be affected clinically (Kaleta and Baldauf 1988). ND is an OIE list A disease agent.

ND virus has a worldwide distribution. However, the widespread use of live ND vaccines, problems with the diagnosis and reporting of ND, and the presence of strains of low virulence to chickens in some countries, makes the assessment of the prevalence of ND difficult (Alexander 2000).

Newcastle disease is highly contagious and transmission of virus is frequently by direct contact with diseased or carrier birds. Virus is excreted from the respiratory tract and in the faeces for at least one day before clinical signs become apparent (Sinha, Hanson, and Brandly 1954) and birds can be infected both by inhalation of aerosols or by ingestion. Infection from fomites such as chicken crates, egg flats, contaminated feed, trucks, dust, humans, other

animals, feathers and clothing is important in the spread of the disease in an outbreak (Lancaster and Alexander 1975; Alexander 2000).

Outbreaks of virulent ND occurred in Australia in 1930, 1932, 1998, 1999, 2000 (Westbury 2001), and 2002. Early outbreaks of ND in Australia were believed to be caused by the feeding of wastes containing viscera of infected birds to backyard flocks (Geering, Forman, and Nunn 1995), while recent outbreaks were due to virulent endemic strains of ND virus closely related to known strains of low virulence (Gould et al. 2001; Westbury 2001). On all occasions, the disease was successfully eradicated by slaughter.

In natural infections, ND virus enters through the respiratory or intestinal tracts and replicates locally. 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 5–6 days).

Large amounts of virus are excreted in the faeces, during the course of infection, and virus is also excreted in the expired air of infected chickens. A long-term carrier state has been postulated for both lentogenic and velogenic ND virus (Heuschele and Easterday 1970).

Both live virus and inactivated vaccines are used in the control of ND. Although vaccination protects birds from the clinical effects of virulent ND, it does not prevent infection with virulent ND virus. 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).

Diagnosis of ND relies on the isolation and characterisation of ND virus. A number of serological tests are available for the diagnosis of ND. At present, the Haemagglutination Inhibition (HI) test is used most widely (Alexander 2000). PMV-1 may show some antigenic cross-reactions in HI tests with PMV-3 and PMV-7 (Alexander 1997), but these can be resolved by the use of suitable antigen and antiserum controls.

Transmission in eggs

ND virus has been demonstrated in and on eggs (Williams and Dillard 1968b; Lancaster 1963) 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, Rhodes-Miller, and Beaudette 1950; Zagar and Pomeroy 1950; French, St George, and Percy 1967; Collins, Gough, and Alexander 1993) but egg transmission of virulent strains has not been considered to be of epidemiological significance as birds quickly cease laying and infected embryos die (Beard and Hanson 1984). Recent studies have shown immune hens challenged with virulent ND virus may lay contaminated eggs. While no virus was isolated from the shell in these studies, challenge virus was isolated from the albumen of one of 187 eggs produced within two weeks of challenge (Australian Animal Health Laboratory 2002).

Egg production may decrease in laying birds infected with virulent ND virus, and eggs may be malformed or have defective shells. Changes in yolk and albumen quality were reported (Monlux 1972).

Conclusions

The “Conditions” require that there is no evidence of illness in the source flock from 90 days prior to egg collection. The source flock may be vaccinated against ND. However, there remains the small likelihood that the external shell of imported eggs may be contaminated, and may lead to infection of hatchlings shortly after hatch. To mitigate this risk, the

“Conditions” require that all hatching eggs are fumigated after collection, and again on arrival in PAQ. This should reduce the likelihood of shell contamination to negligible. Given the highly contagious nature and short incubation period of ND, disease would spread rapidly among the quarantine flock and sentinel birds. If present, ND would be readily diagnosed within the 6 week post-hatch testing period.

Avian Paramyxovirus 2

Avian paramyxoviruses, other than PMV-1 (Newcastle disease virus), exhibit relatively low pathogenicity in domestic poultry, and lower economic impact than ND. Except where infection is complicated by the presence of other pathogens, avian paramyxovirus-2 (PMV-2) infection in poultry has been associated with inapparent or mild respiratory disease. Although infection has been reported in chickens, turkeys, and caged passerine and psittacine birds, the primary natural host appears to be small passerine birds (Alexander 1993). PMV-2 is also known as Yucaipa virus as it was first isolated in Yucaipa, California in 1956 (Bankowski, Corstvet, and Clark 1960).

PMV-2 appears to have a worldwide distribution in various hosts. In poultry, PMV-2 has been isolated from chickens and/or turkeys in countries of North and Central America, Asia, the Middle East and Eastern and Western Europe (Alexander 2000). PMV-2 has not been reported in poultry in Australia or New Zealand.

There are few reports of studies of the transmission of PMV-2. However, since infection of poultry leads to shedding of PMV-2 from the respiratory and intestinal tracts, it is assumed that the methods of spread would be similar to PMV-1. In field infections PMV-2 spreads only slowly through the flock, and flock-to-flock transmission, even between flocks in close proximity, does not always occur (Alexander 1993).

Where infection is uncomplicated by other infections, PMV-2 infection generally results in mild respiratory or inapparent disease(Bradshaw and Jensen 1979).

In field infections of turkeys, seroconversion takes up to 5 weeks, and antibody titres remain low. In some cases, seroconversion occurs only in a proportion of the affected flock (Le Gros 1986). An inactivated, combined ND and PMV-2 vaccine for poultry is available in Israel, together with an inactivated, combined PMV-2 and PMV-3 vaccine for turkeys (Anonymous 2003).

Samples taken and methods used to isolate PMV-2 are the same as those used for ND virus.

Transmission in eggs

There are no reports of transmission of PMV-2 through eggs. However since PMV-2 may be shed from the respiratory and intestinal tracts, it is assumed that contamination of the shell could occur.

Conclusions

The “Conditions” require that there is no evidence of illness in the source flock from 90 days prior to egg collection. Testing of the source flock on two occasions should detect positive birds and/or any increase in antibody titres around the time of egg collection. There is no evidence that PMV-2 is egg transmitted. To mitigate the risk associated contamination of egg shells, the “Conditions” require that hatching eggs are fumigated after collection and again on

arrival in PAQ. This should reduce the likelihood of shell contamination to negligible. Although infection spreads slowly through most flocks, it is considered that a serological test undertaken at 6 weeks post-hatch provides sufficient additional confidence that PMV-2 is not present in the imported birds.

Avian Paramyxovirus-3

Avian paramyxovirus-3 (PMV-3) causes respiratory signs and decreased egg production in infected turkey flocks. Although turkeys are considered to be the primary natural host of PMV-3, experimental studies have shown that chickens are susceptible to infection (Alexander 1997) and there is one report of isolation of PMV-3 from a flock of chickens with respiratory disease (Shihmanter et al. 2000). PMV-3 has also been isolated from captive dead or dying psittacine and passerine birds held in quarantine (Alexander 1997).

Turkeys are considered to be the primary natural host of PMV-3 and most reports of natural PMV-3 infections in domestic poultry are from turkeys in Western Europe, North America and Israel (Alexander 2000). There is one report of isolation of PMV-3 from the cloacal swab of a chicken showing clinical signs of respiratory disease in Israel (Shihmanter et al. 2000). PMV-3 has not been reported in poultry in Australia or New Zealand.

There are few reports of studies of the transmission of PMV-3. Since mild respiratory disease is the earliest sign of infection, the primary site of infection is likely to be the respiratory tract. Virus has been isolated from tracheal and cloacal swabs of domestic poultry, so transmission probably occurs through aerosols and ingestion of material contaminated with faeces. In field infections it is reported that PMV-3 spreads only slowly through the flock, and flock-to-flock transmission, even between flocks in close proximity, does not always occur (Alexander, Pattison, and Macpherson 1983; Alexander 1993).

There are no reports of transovarian transmission of PMV-3 (Alexander 1993).

PMV-3 viruses may show sufficiently high levels of cross reactivity with conventional PMV-1 (ND virus) antisera to cause problems with interpretation of serological tests. Apparent antibodies to PMV-3 in chickens have been attributed to high antibody levels to ND virus as a result of vaccination (Box, Holmes, and Webb 1988). Prior infection of chickens with PMV-3 conferred some protection against challenge with a virulent strain of ND virus (Alexander, Chettle, and Parsons 1979).

Virus isolation is often difficult since the virus is shed for only a short time. The virus may be isolated from swabs or samples of trachea, lung, sinus, cloaca and pharynx inoculated into embryonated chicken eggs.

A number of serological tests are available to assist with diagnosis of PMV-3. At present, the haemagglutination inhibition (HI) test is used most widely (Alexander 2000). PMV-3 may show some antigenic cross-reactions in HI tests with PMV-1 (Alexander 1997), but these can be resolved by the use of suitable antigen and antiserum controls.

Transmission in eggs

There are no reports of transovarian transmission of PMV-3. However since PMV-3 may be shed from the respiratory and intestinal tracts, it is assumed that contamination of the shell could occur.

Conclusions

The "Conditions" require that there is no evidence of illness in the source flock from 90 days prior to egg collection. Testing of the source flock on two occasions should detect positive birds and/or any increase in antibody titres around the time of egg collection. There is no evidence that PMV-3 is egg transmitted. To mitigate the risk associated contamination of egg shells, the "Conditions" require that hatching eggs are fumigated after collection and again on arrival in PAQ. This should reduce the likelihood of shell contamination to negligible. Although infection spreads slowly through most flocks, it is considered that a serological test undertaken at 6 weeks post-hatch provides sufficient additional confidence that PMV-3 is not present in the imported birds.

Turkey Rhinotracheitis/avian pneumovirus

Avian pneumovirus (APV) is a significant respiratory pathogen in both turkey and chicken flocks, causing serious economic losses in birds of any age. In turkeys, initial infection of the upper respiratory tract with APV is frequently complicated by secondary infections with *E. coli*, infectious bronchitis virus, or *Mycoplasma* spp. The disease caused by APV in turkeys is known as turkey rhinotracheitis (TRT) or turkey coryza. APV also infects chickens and is associated with swollen head syndrome in meat chickens and breeders (Cook 2000).

APV occurs in most countries of the world, although reports are frequently based only on serological evidence. Published reports indicate that Canada (Heckert and Meyers 1993) and Australia (Bell and Alexander 1990) are free from APV.

APV is highly infectious, and spreads rapidly once it is introduced into susceptible flocks. Where birds are housed in close proximity, APV spreads rapidly, but spread is slower over greater distances. Given the nature of the infection, transmission is likely to be airborne, although faecal shedding may occur. Transmission by contaminated water, movement of infected birds, and fomites (personnel, vehicles, egg trays) is also possible. APV persists only for short time in the infected bird so shedding is likely to be of short duration only.

Field evidence suggests that transmission of APV through the egg (either transovarially or by egg contamination) is unlikely to occur (Cook 2000). APV has been transmitted experimentally by contact, or by intranasal, ocular or oculonasal inoculation, and there are two reports of transmission via the oral route (Shin et al. 2001; Nagaraja et al. 2002).

Swollen head syndrome has been described in 3- to 5-week-old meat chickens (Gough et al. 1994) and in older meat chicken breeders, but not in commercial egg laying flocks to any extent (Naylor and Jones 1993). The incubation period in experimentally infected 3-week-old chicks was 3 to 6 days (Majó et al. 1995).

Live attentuated and inactivated vaccines are available to control APV infections in turkeys and chickens. Live vaccines are used to control infections in growing turkeys and meat chickens, and to prime future layers and breeders before the injection of inactivated vaccine prior to onset of lay. The duration of immunity following vaccination is at least 14 to 22 weeks. Maternal immunity does not appear to protect against infection, or prevent successful immunisation (Cook et al. 1995).

APV in infective mucus, nasal secretions or sinus scrapings may be isolated in chicken or turkey embryo tracheal organ culture, or in embryonated eggs inoculated via the yolk sac. Due to the short duration of virus shedding, isolation should be attempted at the first sign of clinical disease.

Transmission in eggs

Although APV has been demonstrated in the reproductive tract of experimentally infected laying turkeys, there is no evidence of egg transmission of APV. However, the virus may be shed in faeces and respiratory secretions and thus contaminate the shell of eggs.

Conclusions

The “Conditions” require that there is no evidence of illness in the source flock from 90 days prior to egg collection. Testing of the source flock on one (unvaccinated) or two occasions (if vaccinated against TRT) should detect positive birds and/or any increase in antibody titres around the time of egg collection. There is no evidence that APV is egg transmitted. To mitigate the risk associated contamination of egg shells, the “Conditions” require that hatching eggs are fumigated after collection and again on arrival in PAQ. This should reduce the likelihood of shell contamination to negligible. Although faecal shedding of the virus occurs only for a short period, the “Conditions” require that diagnostic testing be carried out on birds showing evidence of disease in quarantine. It is considered that a serological test on sentinel birds provides sufficient additional confidence that APV is not present in the imported birds.

Salmonella Pullorum *and Salmonella* Gallinarum

Pullorum disease and fowl typhoid are septicaemic bacterial diseases of chickens, turkeys and pheasants. Pullorum disease is caused by *Salmonella* Pullorum, while fowl typhoid is caused by *Salmonella* Gallinarum (Shivaprasad 2000). These two *Salmonella* species are distinguished from the remainder of the salmonellae, in that they are host-adapted and highly pathogenic for chickens and turkeys, but have little public health significance (Wray and Davies 2000).

Both pullorum disease and fowl typhoid are OIE list B diseases. While the OIE records that there is serological evidence of pullorum disease in Australia, these two diseases have been eradicated from Australian commercial flocks (Anonymous 1998b). The Australian Salmonella Reference Laboratory has not recorded isolation of *S.* Pullorum from any Australian source in the last 10 years (Anonymous 2000). Fowl typhoid was last reported in Australia in 1952 (Animal Health Australia 2001).

Chickens are the natural hosts for both *S.* Pullorum and *S*. Gallinarum (Shivaprasad 1997).

Pullorum disease and fowl typhoid are widely distributed throughout the world. The diseases have been eradicated from commercial poultry via a test-and-slaughter method of disease control in the USA, Canada, Australia, Japan and most countries in Western Europe (Shivaprasad 2000; Rabsch et al. 2000; Audisio and Terzolo 2002).

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 1997). 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 (Shivaprasad 1997). Transmission via the egg can also occur through shell penetration but has been reported to be of minor importance (Williams, Dillard, and Hall 1968). 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 1997; Wray and Davies 2000).

Horizontal transmission between chicks or pullets is an important means of dissemination of both organisms. Fluff from infected chicks is heavily contaminated, and bacteria may be disseminated throughout the incubator or brooder leading to lateral transmission between chicks (Wray and Davies 2001). Faeces from infected birds are an important source of infection, while other routes of infection include fomites (contaminated feed, water, litter, attendants), cannibalism and egg eating (Shivaprasad 1997). Wild birds, other animals and flies may spread the organisms mechanically (Shivaprasad 2000), although the importance of wild birds in the spread of these non-motile *Salmonellae* has been questioned (Wilson and MacDonald 1967).

Pullorum disease is principally a disease of chicks and poults, mortality being highest within the first 2-3 weeks of life (Shivaprasad 1997). Mortality and wasting of adult chickens can be caused by pullorum disease (Erbeck, McLaughlin, and Singh 1993). Fowl typhoid, frequently referred to as a disease of growing or adult birds, can cause clinical signs in young chickens that are indistinguishable from those seen in pullorum disease (Wray and Davies 2000). Mortality rates in chicks of up to 26% have been recorded during the first month of life in association with fowl typhoid (Shivaprasad 1997). Mortality due to pullorum disease and fowl typhoid often begins in the hatchery, but with fowl typhoid the mortalities may persist to laying age (Shivaprasad 1997).

The incubation period of *S*. Pullorum varies with the route of infection and the age and condition of the host. With egg-transmission, moribund and dead chicks may be found in the incubator shortly after hatching, although in some cases signs of disease are not seen for 5-10 days after hatching. n adult birds, body temperature may increase within 2-3 days of exposure, followed by clinical signs and possibly death within 4-10 days (Shivaprasad 1997). Clinical signs of fowl typhoid were seen at 3-6 days after oral inoculation in one study (Mdegela et al. 2002), but not until 8 days post-inoculation in another (Jones et al. 2001).

Transmission in eggs

S. Pullorum is vertically transmitted, with organisms localising in the ovule or contaminating the ovum following ovulation.

There is conflicting evidence regarding egg transmission of *S*. Gallinarum. Although vertical transmission of *S*. Gallinarum is reported (Shivaprasad 1997; Wray and Davies 2001), recent attempts to isolate the organism from the eggs of experimentally-infected hens were unsuccessful (Berchieri et al. 2001). Furthermore, in an *in vitro* experiment, *S*. Gallinarum was isolated from eggs immediately after inoculation with the organism, but could not be isolated from the eggs 24 and 48 hours later (Berchieri et al. 2001). Therefore, the role of vertical transmission in the spread of *S*. Gallinarum is unclear.

Conclusions

Testing for *Salmonella* organisms is currently conducted in the first 10 days post-hatch, and will therefore be unaffected by the proposed change in the quarantine conditions.

Salmonella Enteritidis

S. Enteritidis is a non-host-specific bacterial pathogens, principally of concern as a major cause of food-borne salmonellosis in humans. In poultry, strains of *Salmonella* Enteritidis cause systemic infection, leading to contamination of meat and eggs. *S*. Enteritidis seldom causes clinical disease, except in susceptible young birds (Gast 1997). The serovar is distributed virtually worldwide in a range of species (Barrow 2000) but significant subtypes and particularly strains of concern are not endemic in Australian poultry flocks.

In general, *S*. Enteritidis does not cause severe systemic disease in physiologically and immunologically normal, healthy adult chickens (Barrow 2000). Clinical disease usually occurs only in very young birds, and birds infected within 24 hours of hatching may develop persistent infection and shed organisms for up to 28 weeks.

Transmission is generally by mouth, but infection may also occur by the respiratory or conjunctival route, via the egg (transovarian) and via the cloaca. The organisms are shed in relatively large numbers in the faeces. The duration of faecal excretion is affected by the external temperature, the use of antibiotics and growth promoters, and concomitant infections with other agents such as IBDV and *Eimeria* (Barrow 2000). 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).

Transmission in eggs

Intact shell eggs have been implicated as the major vehicle of transmission of *S*. Enteritidis in a number of countries (cited by Cox 1995).

Conclusions

Testing for *Salmonella* organisms is currently conducted in the first 10 days post-hatch, and will therefore be unaffected by the proposed change in the quarantine conditions.

Salmonella Arizonae

The name *Salmonella* Arizonae is used to designate a group of bacteria comprising some 415 different antigenic types (Davos 2001). Although infections have been reported in sheep, cattle, reptiles and birds, the organism is most commonly isolated from reptiles and turkeys (Shivaprasad et al. 1997).

Although reports of arizonosis in chickens are few, evidence suggests that serious disease could result if the organism becomes established in chickens (Silva, Hipolito, and Grecchi 1980).

S. Arizonae is non-host specific and occurs worldwide. Among poultry, *S*. Arizonae is most frequently isolated from turkeys, but chickens are affected both experimentally and naturally. Ducks are also susceptible. Wild birds and reptiles are potential sources of infection in poultry houses.

Infected adult birds may carry the organism in their intestine for prolonged periods and thus spread the disease. *S*. Arizonae has been isolated from soil, feed, litter, or eggshell (Geissler and Youssef 1981) and may be transmitted in contaminated feed and water. The organism may also be spread in the incubator and brooder by direct contact.

There are few reports of the prevalence of avian arizonosis. The organism has been eliminated from turkey flocks in the UK by a rigorous program of slaughter of the original breeding flock, selection, management, screening and culling of progeny flocks (Shivaprasad et al. 1997).

Confirmation of the diagnosis depends on isolation and identification of *S*. Arizonae from infected birds. Culture of shells and shell membrane of turkey egg allows rapid detection of contaminated eggs (Greenfield, Bigland, and Dukes 1971).

Transmission in eggs

S. Arizonae has been demonstrated in the contents of both chicken and turkey eggs. Although transovarian transmission may occur, this is not common and it is considered more likely that contamination of the contents is due to penetration of the cuticle, shell, inner and outer shell membranes of intact eggs by the organism. Eggshell penetration by *S*. Arizonae has been shown to occur experimentally in around 5% of chicken eggs (Williams and Dillard 1968a).

Conclusions

Testing for *Salmonella* organisms is currently conducted in the first 10 days post-hatch, and will therefore be unaffected by the proposed change in the quarantine conditions.

Mycoplasma iowae, Runting Stunting Syndrome and *Ornithobacterium rhinotracheale*

The "Conditions" do not require testing of hatched or sentinel birds in PAQ for these diseases or disease agents.

Recommendations

When the "Conditions" were developed, the length of the PAQ period was set at 12 weeks:

- to ensure that sufficient time had elapsed post-hatch for any infection in the quarantine birds to spread to more individuals in the quarantine flock, or to the sentinels;
- to ensure that sufficient time had elapsed for clinical signs to be shown by the quarantine or sentinel birds;
- to ensure that sufficient time was available for all required testing to be undertaken, and for results of that testing to become available.

From the preceding discussion, it is clear that the PAQ could be shortened to a total of 9 weeks post-hatch without significantly increasing the risk of introducing exotic pathogens of quarantine concern into Australia, provided all testing of parent flocks is carried out and test results are received and notified in a timely manner.

In addition to the requirements for general observation of source flock health over the period before and after egg collection, it is recommended that the "Conditions" be modified as suggested in Table 2 below.

Table 2: Recommended changes to testing requirements for hatching eggs of domestic hens

Type of test	Timing				
	PEQ		***PAQ***		
	< 21 days before egg collection.	***14 – 21 days post egg collection***	***0 – 10 days post hatch***	***6 weeks post hatch***	***9 weeks post hatch***
Serology (parent flock)	AI, ND, PMV2, PMV3, TRT, *Salmonellae*	AI, ND, TRT (if vaccinated)			
Serology (sentinels)				AI, ND, TRT, IBD *Salmonellae*	
Agent isolation (wastes, litter etc)	*Salmonellae* in shed litter		*Salmonellae* in hatchery waste, dead birds, healthy culls, shed litter		
Agent isolation (quarantine birds)				AI, ND, PMV2, PMV3, IBD	

Existing policy (Domestic turkeys)

The current "CONDITIONS FOR THE IMPORTATION FROM APPROVED COUNTRIES OF FERTILE EGGS (DOMESTIC TURKEY)" define the following as diseases for the purposes of the "Conditions":

Arizona disease (*Salmonella* Arizona)

Avian influenza

Avian paramyxoviridae type 2 and 3 infection

Fowl typhoid (*Salmonella* Gallinarum)

Infectious bursal disease

Mycoplasma iowae infection

Newcastle disease

Ornithobacterium rhinotracheale infection

Pullorum disease (*Salmonella* Pullorum)

Runting/stunting syndrome

S*almonella* Enteritidis infection

Turkey Lymphoproliferative disease

Turkey meningoencephalitis

Turkey viral hepatitis

Turkey viral rhinotracheitis

A copy of the "Conditions", describing the import conditions and testing requirements in detail, is included as Appendix 5.

Current testing regime

In addition to the requirements for general observation of source flock health over the period before and after egg collection, the "Conditions" set out a detailed program of testing designed to identify even low-level infection in the source and quarantine flocks with a number of the diseases of quarantine concern. Note that specific testing for runting stunting syndrome, *Ornithobacterium rhinotracheale*, turkey lymphoproliferative disease, turkey meningoencephalitis, turkey viral hepatitis and turkey viral rhinotracheitis is not currently required. However, these diseases would be detected on investigation of morbidity or mortality (including reduced hatchability) in the source and quarantine flocks, as required in the "Conditions".The current testing regime is summarised in Table 3 below.

Table 3: Current testing requirements for hatching eggs of domestic turkeys

Type of test	Timing				
	PEQ		***PAQ***		
	< 21 days before egg collection.	***14 – 21 days post egg collection***	***0 – 10 days post hatch***	***6 weeks post hatch***	***9 weeks post hatch***
Serology (parent flock)	AI, ND, PMV2, PMV3, TRT, *Salmonellae*	AI, ND, TRT (if vaccinated)			
Culture (parent birds)	*M. iowae*				
Serology (sentinels)				IBD	IBD AI, ND, *Salmonellae,* TRT
Agent isolation (wastes, litter etc)	*Salmonellae* in shed litter		*Salmonellae* in hatchery waste, dead birds, healthy culls, shed litter		
Agent isolation (quarantine birds)					AI, ND, PMV2, PMV3, IBD

There are no significant differences between the post arrival testing regimes for domestic chickens and domestic turkeys. Additional testing of parent birds for *Mycoplasma iowae* is undertaken in the pre-egg collection period, but this will not impact on post arrival quarantine periods. Moving the sample collection and testing forward from 9 weeks post-hatch to 6 weeks post-hatch should not increase the risk associated with the import of hatching eggs of domestic turkeys.

Recommendations

It is recommended that the “CONDITIONS FOR THE IMPORTATION FROM APPROVED COUNTRIES OF FERTILE EGGS (DOMESTIC TURKEY) be amended in the same way as the “CONDITIONS FOR THE IMPORTATION FROM APPROVED COUNTRIES OF FERTILE EGGS (DOMESTIC HEN)”.

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