Hatching eggs of domestic hens and turkeys—avian paramyxovirus 2 and 3

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Summary

The Australian Government Department of Agriculture has statutory authority for issuing import permits for goods. In doing so, the department assesses the level of any biosecurity risks and develops measures to manage them. These measures may be reviewed if the risk profile of existing trade in a good or that of a pest or disease has changed or may change.

Hatching (fertile) eggs of domestic hens and turkeys from approved countries are imported into Australia under existing biosecurity policy. This policy was last reviewed in 2004 which resulted in a reduction of the post-arrival quarantine period.

Stakeholders have expressed interest in the department continuing to update the Conditions for the Importation of Fertile Eggs (Domestic Hen) and the Conditions for the Importation of Fertile Eggs (Domestic Turkey). Since the policies were revised in 2004 and 2009 new scientific information has become available on avian paramyxoviruses (APMV). In response, the department has conducted this review of the biosecurity risks to Australia by the importation of hatching (fertile) eggs of domestic hens and turkeys from approved countries with respect to avian paramyxovirus 2 (APMV-2) and avian paramyxovirus 3 (APMV-3). It examines risk management options to reduce identified risks to a level consistent with Australia's appropriate level of protection (ALOP).

A draft policy review was released for 60 days public comment to allow stakeholders time to assess and comment on the proposed changes. Stakeholder submissions were considered when finalising the review.

This policy review concludes that there is little or no evidence that APMV-2 is pathogenic to chickens or turkeys. Therefore APMV-2 does not qualify as a hazard as defined in the World Organisation for Animal Health Terrestrial Animal Health Code (the Code) and risk management is not required. The following recommendation is made:

- the requirements for testing for APMV-2 be deleted from both the Conditions for the Importation of Fertile Eggs (Domestic Hen) and the Conditions for the Importation of Fertile Eggs (Domestic Turkey).

For APMV-3, this policy review concludes that there is evidence that APMV-3 is pathogenic to turkeys and is therefore a hazard as defined by the Code. Although APMV-3 is not a natural infection of chickens, they may be infected experimentally and could introduce APMV-3 into Australia with subsequent spread to the Australian turkey flock. After consideration of submissions received from stakeholders, the risk posed by APMV-3 risk has been re-assessed with the conclusion that the biosecurity risk of this agent is above the level set as Australia's Appropriate Level of Protection (ALOP) of 'very low' and therefore requires risk management. The following recommendation is made:

- requirements for testing for APMV-3 be retained in the Conditions for the Importation of Fertile Eggs (Domestic Hen) and the Conditions for the Importation of Fertile Eggs (Domestic Turkey).
Full details of the risk assessment and the conclusions reached for APMV-2 and APMV-3 are provided in this policy review.
1. Introduction

1.1 Background

Hatching (fertile) eggs of domestic hens and turkeys from approved countries are imported into Australia under existing biosecurity policy.

Stakeholders have expressed interest in the Australian Government Department of Agriculture (the department) continuing to update the Conditions for the Importation of Fertile Eggs (Domestic Hen) and the Conditions for the Importation of Fertile Eggs (Domestic Turkey) with respect to the risk management requirements for specific agents.

Consequently, in this policy review the department has re-examined the risk management measures for avian paramyxovirus 2 (APMV-2) and avian paramyxovirus 3 (APMV-3) with due regard to Australia’s appropriate level of protection (ALOP). These viruses are related to but different from Newcastle disease virus (NDV) which is designated avian paramyxovirus 1 (APMV-1). Other disease agents will be considered in future reviews.

1.2 Australia’s biosecurity policy

Australia’s biosecurity policies aim to protect Australia from risks that may arise from exotic diseases and pests entering, establishing and/or spreading, thereby threatening Australia’s unique flora and fauna, as well as agricultural industries that are relatively free from serious diseases and pests.

The department is responsible for developing and reviewing biosecurity policy for the import of animals and their products. It does this through a science-based risk analysis process. At the completion of the process and following consideration of stakeholder comments, recommendations are made to Australia’s Director of Animal and Plant Quarantine who is responsible for determining whether or not imports can be permitted under the Quarantine Act, 1908 and if so, under what conditions.

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

Australia implements a risk-based approach to biosecurity management. This approach is expressed in terms of Australia’s ALOP, which reflects community expectations through government policy and is currently aimed at reducing these risks to a very low level, but not to zero.

If the risks exceed Australia’s ALOP, risk management measures are proposed to reduce the risks to an appropriate level. However, if it is not possible to reduce the risks to an appropriate level, then no trade will be allowed.
1.3 Scope

This policy review considers the biosecurity risks that may be associated with two paramyxoviruses—avian paramyxovirus 2 (APMV-2) and avian paramyxovirus 3 (APMV-3) related to the importation into Australia of hatching eggs of domestic hens (chickens) and of turkeys from approved countries. Under the current import requirements the approved countries are Canada, France, Germany, Ireland, Netherlands, New Zealand, United Kingdom and United States of America.

1.4 Current import conditions

Import requirements for hatching eggs of domestic hens and turkeys were established in 1989. These require isolation and testing of source flocks in the source countries, treatment of the imported eggs and importation into an approved post arrival quarantine (PAQ) facility. Further testing is carried out after the quarantine flock has hatched, both of the quarantine flock itself and of specific pathogen free (SPF) chickens hatched as sentinels.

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. In 2005 the import requirements for hatching eggs of hens and turkeys were amended in response to outbreaks of highly pathogenic H5N1 avian influenza in many parts of the world.


1.5 Potentially affected Australian industries

The introduction of an exotic avian disease could potentially affect several Australian industries or groups:

- commercial chicken meat industry
- commercial egg industry
- commercial turkey industry
- other
  - aviculture community
  - commercial duck industry
  - game birds, e.g. pheasant, guinea fowl
  - native birds and the environment
  - pigeons
  - ratite industry
  - zoos.
1.6 Current risk management measures for APMV-2 and APMV-3

1.6.1 Conditions for the importation of fertile eggs (Domestic Hen)

APMV-2 and APMV-3 are listed as ‘diseases’ in Section 5 (f) of the Conditions for the importation of fertile eggs (Domestic Hen) dated 11 November 2005.

The risk management measures are:

- the source flock is required to be free of clinical signs of both agents, and not have come into contact with any birds showing signs of these diseases, for the 90 day period prior to collection of eggs for export to Australia
- no floor or dirty eggs are included in the consignment of eggs for export
- after collection the eggs are fumigated or disinfected
- in the 21 days before egg collection for export commences, the source flock is tested serologically for antibodies against APMV-2 and APMV-3. The sample tested is of sufficient size to give a 99% confidence of detecting 0.5% disease prevalence. This is the same detection level as for a number of other agents of biosecurity concern e.g. avian pneumovirus, if the flock has not been vaccinated against this agent, and Salmonella species
- the source flock is required to be free of disease caused by both agents during the period of the collection of eggs for export to Australia
- on arrival at the quarantine station in Australia the eggs are fumigated or disinfected
- cloacal swabs are taken from a sample of the PAQ flock sufficient to give a 99% confidence of detecting 5% disease prevalence in the flock and the samples are tested for haemaggultinating agents. This testing will detect the presence of APMV-2 and APMV-3.

There is the option in the Conditions for the importation of fertile eggs (Domestic Hen) that an exporting country authority may claim country freedom for these agents. There is also the option of placing Newcastle disease virus (NDV) sero-negative sentinel chickens in a source flock that has been vaccinated against Newcastle disease. In this case the sentinels are tested twice—the first time for Newcastle disease and again, after the eggs have been collected, for Newcastle disease, APMV-2 and APMV-3.

1.6.2 Conditions for the importation of fertile eggs (Domestic Turkey)

APMV-2 and APMV-3 are listed as ‘diseases’ in Section 5 (f) of the Conditions for the importation of fertile eggs (Domestic turkey) dated 11 November 2005.

Risk management measures are identical to those for domestic hens and include options for country freedom and for the use of NDV sero-negative turkeys within the source flock. However, some turkey flocks supplying eggs for export to Australia have been vaccinated against APMV-3 and in these cases, equivalence has been granted by requiring 100 individually identified birds from the source flock to be tested twice for APMV-3—the first time before egg collection commences and again following the
completion of egg collection. This is the same regime for Newcastle disease testing of vaccinated flocks.

2. Risk reviews

The risks associated with APMV-2 and APMV-3 were assessed according to the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code (the Code), Chapter 2.1 Import Risk Analysis. In this chapter, a risk analysis is defined as comprising of hazard identification, risk assessment and risk management, all supported by risk communication (OIE 2012a).

Hazard identification identifies pathogenic agents that have the potential to produce adverse consequences in the importing country. The hazard must be appropriate to the species to be imported and present in the exporting country while not present, or subject to official control, in the importing country.

Risk assessment is described as having three steps—assessment of the likelihood of entry, of the likelihood of exposure of the relevant population in the importing country should entry occur and the consequences of that exposure. These will lead to an estimation of the overall risk posed by the hazard.

Risk management is the process of deciding upon and implementing measures to achieve the importing country's appropriate level of protection (ALOP).

2.1 Avian paramyxovirus 2

2.1.1 Technical information

Background

Avian paramyxovirus 2 (APMV-2) is one of at least 10 and probably 11 identified avian paramyxovirus serotypes, the most significant being APMV-1 (Newcastle disease virus; NDV) (OIE 2012b). Avian paramyxoviruses are members of the genus Avulavirus of the family Paramyxoviridae (Büchen-Osmond 2008). Other than NDV, these agents have received little attention due to their relatively low pathogenicity in domestic poultry and low economic impact.

APMV-2 is not an OIE-listed disease, is not a nationally notifiable disease in Australia and is not known to be a human pathogen. APMV-2 has not been isolated from avian species in Australia.

Agent characteristics

Avian paramyxoviruses are enveloped ribonucleic acid (RNA) viruses and are destroyed rapidly outside the host species. In general, paramyxoviruses are sensitive to thermal inactivation, lipid solvents and chlorine-based disinfectants.
In the absence of any specific reports on the inactivation of APMV-2, it is assumed that it has a similar spectrum of sensitivity to NDV (Alexander and Senne 2008a). Patnayak et al. (2008) showed that NDV was most sensitive to phenols and glutaraldehyde but resistant to quaternary ammonium compounds. Quinn and Markey (2001) classified paramyxoviruses in Viral Susceptibility Group A—susceptible to alcohols, aldehydes, detergents, halogens, H$_2$O$_2$, phenolics, proteases and quaternary ammonium compounds.

There is considerable antigenic and structural diversity among APMV-2 isolates (Mahmood et al. 2010). Although cross-reactions may occur, APMV-2 is serologically distinct from NDV.

**Epidemiology**

APMV-2 is also known as Yucaipa virus as it was first isolated in Yucaipa, California in 1956 (Bankowski et al. 1960). Although this initial isolation was from chickens, turkeys and passerine birds are considered to be the natural hosts of APMV-2 (Alexander and Senne 2008a).

APMV-2 has been isolated from chickens and/or turkeys in North and Central America, Asia, the Middle East, and Eastern and Western Europe (Alexander 2000). APMV-2 has not been reported in poultry in Australia or New Zealand.

There are few reports of the prevalence of APMV-2 in poultry. A serological survey carried out in the United States in 2008 attempted to establish the prevalence of APMV-2 in chickens (Warke et al. 2008a). Although sera from 10 of 47 flocks of broiler breeders and 3 of 29 flocks of egg layers were positive, all these flocks were also positive to NDV. Although cross-reactions between NDV and APMV-2 are generally not considered likely to occur, Warke et al. (2008a) concluded that cross reactions between NDV and all the other avian paramyxoviruses were likely if the NDV titres were high enough. The prevalence of APMV-2 in these flocks was estimated to be less than 10% but the issue of cross-reactivity with NDV remained unresolved (Warke et al. 2008a).

These findings throw into doubt the results of the few serological surveys of poultry that have been published. In Spain, 14.7% of layer hens (50 of 341 birds sampled) on 43.7% of farms surveyed (21 of 44 farms), and 39% of meat chickens (48 of 123 birds sampled) from 80% of farms surveyed (4 of 5 farms) were shown to be serologically positive for APMV-2 antibodies (Maldonado et al. 1994). However the NDV status of these flocks was unknown and it is likely that a proportion of these results were cross-reactions. An earlier survey carried out in the United States demonstrated similar results but again, the prevalence of NDV was unknown (Bradshaw and Jensen 1979). Bankowski et al. (1968) surveyed both chickens and turkeys and found a higher prevalence in turkeys (27 of 249 positive) than in chickens (4 of 253 positive birds) from 169 farms. However of the 27 turkeys positive to APMV-2, 26 were also positive to NDV, again raising the possibility of cross-reactivity.

There are few reports of studies on the transmission of APMV-2. Alexander and Senne (2008a) state that infection with APMV-2 leads to shedding from the respiratory and intestinal tracts. In field infections, APMV-2 spreads only slowly through the flock and
not all birds may show a serological response (Le Gros 1986). Flock-to-flock transmission, even between flocks in close proximity, does not always occur (Alexander 1993).

It has been postulated that wild passerine birds are responsible for spread to other species by contact or invasion of poultry houses (Ozdemir et al. 1990).

**Clinical signs**

Generally it has been accepted that APMV-2 results in mild respiratory or inapparent disease when infection is uncomplicated by the presence of other agents. However, instances of uncomplicated infections are rare.

The initial isolation of APMV-2 was from chickens suffering from severe infectious laryngotracheitis (Bankowski et al. 1960) and all reported isolations associated with disease since have also been in conjunction with other agents able to cause the disease symptoms seen in their own right. Isolations in Canada in 1975 were from turkeys showing respiratory signs and mortality but these flocks were also infected with mycoplasma, *Salmonella Arizona* and fowl cholera (*Pasteurella multocida*) (Lang et al. 1975). High morbidity and mortality has been reported in Israel in turkeys positive for APMV-2, but these flocks were also infected with fowl cholera, mycoplasma and turkey coryza (Lipkind et al. 1982). An APMV-2 isolate was also recovered at this time from a duck that, along with a number of others, had died of avian influenza.

While no specific disease agents were identified in an investigation of respiratory disease in turkeys in California in 1979, it was concluded that the APMV-2 virus identified by serology and isolation from tracheal swabs was not the cause of the clinical signs (Bradshaw and Jensen 1979). Non-specific disease signs have been reported in finches from which APMV-2 viruses were isolated and APMV-2 has been isolated from ‘sickened’ chickens but there was no indication as to the range or severity of symptoms, or what other agents may have been present (Zhang et al. 2006). The exception is a conference report which describes mortality and morbidity in turkeys and chickens due to both APMV-2 and APMV-3 (Weisman et al. 1999). No further information on this apparent outbreak has been published and there are no details on whether other agents were involved.

Experimental infections of APMV-2 indicate that there is little or no pathology or clinical signs produced when poultry is infected by this agent alone. Early challenges by intratracheal infection were reported to elicit only very mild upper-respiratory symptoms (Lang et al. 1975). However, in an early study APMV-2 was embryo-lethal when inoculated into eggs (Bankowski and Corstvet 1961).

However, subsequent studies have not confirmed this lethal effect on embryos. Subbiah et al. (2010) experimentally infected nine day-old embryos, one day-old SPF chicks, four week old SPF chickens and four week-old SPF turkeys. The Mean Death Time (MDT)\(^1\) in embryos in this study was >168 hours and the Intra-Cerebral Pathogenicity Index

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\(^1\) see method in Alexander and Senne 2008a
in one day-old SPF chicks was zero, indicating that the agent was non-pathogenic. The SPF chickens and turkeys were inoculated intranasally and seroconverted but no disease was observed. Another study in 2012 established a MDT for embryos over >144 hours and an ICPI for day-old chicks of zero, thereby reinforcing the avirulent nature of APMV-2 (Kim et al. 2012).

High doses of APMV-2 inoculated intranasally into day old SPF chicks produced mild diarrhoea but no respiratory signs (Warke et al. 2008b).

Pathogenesis

There are few studies on the pathogenesis of APMV-2. In one study, following inoculation of day old chicks with APMV-2, replicating virus was detected in only a few chicks. At four days post-inoculation, virus was isolated from the trachea and lung of two out of five chicks sampled. At seven days post-inoculation, virus was isolated from a single intestinal sample and then nothing until day 28 when virus was isolated from the pancreas of two chicks (Warke et al. 2008b).

Virus was found to be widely distributed through the body, including the respiratory and gastrointestinal systems, and in one case, brain, following experimental infection of 4-week old SPF chickens and turkeys (Subbiah et al. 2010).

Another study showed that when APMV-2 was inoculated intranasally into day-old and two-week-old specific pathogen free (SPF) chickens, replication of the agent was restricted to the trachea. The virus could not be detected in the brain or spleen (Kim et al. 2012).

Pathology

Experimental intranasal inoculation of APMV-2 led to mild lymphocytic tracheitis and mild lung changes (Kim et al. 2012). A similar experiment produced mild catarrhal tracheitis but as control birds were also affected, this finding may have been due to the environmental conditions (Warke et al. 2008b). Experimental infection of four-week old SPF chickens and turkeys produced no overt clinical signs or any gross visceral pathological lesions (Subbiah 2012).

Diagnosis

The samples taken and methods used to isolate APMV-2 are the same as those used for isolation of NDV (OIE 2012b). In addition, inoculation of six- to seven-day-old embryonated eggs via the yolk sac may be used for virus isolation.

Serology

The haemagglutination inhibition test has been used to identify all avian paramyxoviruses except APMV-5 and cross reactions between serotypes are possible (Alexander and Senne 2008b).
Transmission via eggs

There is no evidence that APMV-2 can be transmitted vertically. It is possible that it may be present in faeces and be a contaminant on the eggshell.

Biosecurity significance

APMV-2 is not an OIE-listed disease agent. APMV-2 infection is not notifiable in any state or territory of Australia and is not subject to official controls. APMV-2 is not included in the Emergency Animal Disease Response Agreement.

Conclusion

- The prevalence of APMV-2 in poultry in the countries approved to export hatching eggs to Australia is unknown but is likely to be less than previously assumed (Warke et al. 2008a).
- It is possible that APMV-2 may be present in faeces and be a contaminant on the eggshell. However, there is no evidence that APMV-2 is transmitted via eggs.
- It has been postulated that the introduction of APMV-2 into poultry flocks is by contact with wild passerine birds (Alexander 1986).
- APMV-2 is not a robust organism and is inactivated by a number of common disinfectants (Quinn and Markey 2001). The current import conditions require the surface of imported eggs to be disinfected twice—at egg collection and at arrival at the quarantine station. This will inactivate any APMV-2 in the event it is a contaminant on the outside of the shell.
- There is a low rate of APMV-2 spread between birds and between flocks (Bankowski et al. 1981).
- There is little or no evidence that the agent itself is pathogenic to chickens or turkeys. Reports of morbidity and mortality described as being associated with the isolation of APMV-2 can be wholly ascribed to other pathogenic agents identified at the same time. Experimentally the agent causes mild pathology and minimal if any clinical signs (Bradshaw and Jensen 1979).

2.1.2 Risk assessment

The department concluded that there is little or no evidence that the agent is pathogenic to chickens or turkeys. Therefore, APMV-2 does not qualify as a hazard as defined in the Code and risk management is not required.

2.1.3 Recommendations

The requirements for testing for APMV-2 be deleted from both the Conditions for the Importation of Fertile Eggs (Domestic Hen) and the Conditions for the Importation of Fertile Eggs (Domestic Turkey).
2.2 Avian paramyxovirus 3

2.2.1 Technical information

Background

As for avian paramyxovirus 2, avian paramyxovirus 3 (APMV-3) is one of at least 10, and probably 11, identified avian paramyxovirus serotypes, the most significant being APMV-1 (Newcastle disease virus) (OIE 2012b). Avian paramyxoviruses are members of the genus Avulavirus of the family Paramyxoviridae (Büchen-Osmond 2008).

APMV-3 is not an OIE-listed disease, is not a nationally notifiable disease in Australia and is not known to be a human pathogen. APMV-3 has not been isolated from avian species in Australia.

Agent characteristics

Avian paramyxoviruses are enveloped RNA viruses and are destroyed rapidly outside the host species. In general, paramyxoviruses are sensitive to thermal inactivation, lipid solvents and chlorine-based disinfectants. In the absence of any specific reports on the inactivation of APMV-3, it is assumed that it has a similar spectrum of sensitivity to NDV (Alexander and Senne 2008a). Patnayak et al. (2008) showed that NDV was most sensitive to phenols and glutaraldehyde but resistant to quaternary ammonium compounds. Quinn and Markey (2001) classifies paramyxoviruses in Viral Susceptibility Group A—susceptible to halogens, aldehydes, quaternary ammonium compounds, phenolics, alcohols, H₂O₂, proteases and detergents.

Epidemiology

APMV-3 was first isolated from turkeys in Canada in 1967, then in Wisconsin in the United States in 1968 (Tumova et al. 1979).

Turkeys are considered to be the primary natural host of APMV-3 and most reports of natural APMV-3 infections in domestic poultry are from turkeys in Western Europe, North America and Israel (Alexander 2000). Turkeys may be naturally infected without apparent effects (Alexander et al. 1983). There is a single report of isolation of APMV-3 from the cloacal swab of a chicken in Israel (Shihmanter et al. 2000). Antigenically different strains of APMV-3 have also been isolated from captive birds, principally imported exotic caged psittacine and passerine birds in Asia and Europe and farmed ostriches in Namibia, indicating a wide host range for the virus but there are no reports of isolation of APMV-3 from wild birds (Alexander 1986; Alexander 2000; Kumar et al. 2010).

There are few reports of the prevalence of APMV-3 in poultry and they are complicated by the well recognised cross-reactivity with NDV in serological tests (OIE 2011). Testing of flocks in the United States around the time of the initial isolation of APMV-3 identified serological reactions in four turkey flocks, two at least of which had no NDV antibodies.
(Tumova et al. 1979). At the same time, a chicken flock and wild waterfowl were negative for AMPV-3 (Tumova et al. 1979).

In Britain in the early 1980s, 35% of turkey flocks (12 of 34) had serological reactions to anti-sera from a United States isolate (Alexander et al. 1983). In Spain, 36% of layer hen farms (16 of 44) and 30% of turkey flocks (14 of 47) were serologically positive for APMV-3 antibodies (Maldonado et al. 1994). In both studies, the authors noted a highly significant correlation between APMV-3 and vaccine-induced NDV antibodies. A study of avian paramyxoviruses in chickens in the United States found an initial prevalence of 35% but noted that cross reactions between NDV and all the other avian paramyxoviruses were likely if NDV titres due to vaccination were high enough. Only two of 38 low-NDV titre flocks were positive for APMV-3 and both were adult breeder flocks (Warke et al. 2008b).

In field infections, it is reported that APMV-3 spreads slowly through the flock and flock-to-flock transmission, even between flocks in close proximity, does not always occur (Alexander et al. 1983; Alexander 1993).

**Clinical signs**

The first isolations of APMV-3 were from adult turkey breeder flocks in North America showing large egg production drops, depression and respiratory signs (Tumova et al. 1979). Early isolations in Europe were also associated with egg production problems but usually without respiratory symptoms (Alexander 1993; Andral and Toquin 1984; Le Gros 1986). However, laboured breathing in some turkeys associated with large egg production drops has been demonstrated (Macpherson et al. 1983).

Respiratory disease has been reported in younger turkeys (two to three weeks old) including coughing, nasal discharge and swollen facial sinuses (Redmann et al. 1991). A psittacine strain of APMV-3 was isolated from turkey flocks of unspecified age in Israel with signs of respiratory disease and post-mortem changes including pericarditis, perihepatitis, air sacculitis and pneumonia (Shihmanter et al. 2000). However, *Chlamydophila psittaci* and *E. coli* were also isolated at the same time and they could account for the ante- and post-mortem signs observed.

A single APMV-3 isolate was obtained from one chicken via a cloacal swab although it is unclear what clinical signs this bird or flock were exhibiting (Shihmanter et al. 2000). This followed an earlier, unverified report of disease in turkeys and chickens in Israel associated with the isolation of both APMV-2 and APMV-3 (Weisman et al. 1999).

One of the early United States isolates from turkeys was associated with a drop in egg production along with mild coughing in two experimentally infected adult turkeys (Tumova et al. 1979). An isolate from turkeys in Britain proved lethal when given to one-day old chickens and turkeys by a variety of routes (intravenous, intra-nasal and subcutaneous) but older birds of both species were largely refractory. When non-infected birds were placed in contact with birds inoculated with APMV-3, half of the in-contact turkeys and none of the in-contact chickens seroconverted (Russell et al. 1989).
However in a more recent study when nine-day-old chicken embryos, day-old SPF chickens and turkeys, and two week-old chickens and turkeys were experimentally infected with two strains of APMV-3 (a turkey and a psittacine strain) there was only mild pathogenicity observed. The MDT determinations in chicken embryos and the ICPI determinations in day-old chickens were consistent with a lentogenic (non-virulent) virus. The day-old chickens and turkeys inoculated intra-nasally demonstrated mild respiratory and gastrointestinal signs but no clinical signs were observed in the two week old chickens and turkeys (Kumar et al. 2010).

Lethargy, respiratory distress and deaths were reported in day-old chickens experimentally infected with a strain of APMV-3 isolated from psittacine birds (Alexander and Collins 1982). In contrast a psittacine strain of APMV-3 inoculated into day-old chickens in another study produced no signs of disease or deaths and was described as non-virulent (Kim et al. 2012).

Cachexia and diarrhoea have been reported in passerine birds, and weakness, anorexia, vomiting and sneezing in psittacine birds positive for APMV-3 (Shihmanter et al. 1998). Infection has also been associated with neurological signs and high mortality in captive psittacine birds (Jung et al. 2009).

**Pathogenesis**

Experimental infection in young chickens and turkeys leads to systemic infection—virus was detected in brain, lung, spleen, trachea, pancreas and kidney—but no clinical disease. In general, turkeys are less affected in terms of spread and duration of infection than chickens (Kumar et al. 2010).

Following intranasal inoculation APMV-3 replicated to moderate levels in all the tissues examined (trachea, lung, spleen and the brain) from one-day-old chickens. In two-week old chickens in the same study, viral replication was detected in the trachea and brain, indicating that it is still neurotropic in older chickens despite the restricted replication. However it was not neurovirulent and the study concluded that this psittacine strain of APMV-3 had approximately the same pathogenicity for chickens as the La Sota strain (a lentogenic vaccine strain) of Newcastle disease virus (Kim et al 2012).

**Pathology**

Experimental infection in young chickens and turkeys causes changes in the respiratory tract—air sacculitis, conjunctivitis, laryngitis and rhinitis in both inoculated and in-contact birds (Redmann et al. 1991). Another experimental infection of young birds produced focal pancreatic necrosis but no lesions in the respiratory tract (Kumar et al. 2010). Examination of AMPV-3 infected two-week-old chickens identified tracheitis and mild pathology of the respiratory tract (Kim et al. 2012).

**Diagnosis**

The samples taken and methods used to isolate APMV-3 are the same as those used for isolation of NDV. In addition, inoculation of six- to seven-day-old embryonated eggs via
the yolk sac may be used. APMV-3 may also be cultured in chicken embryo kidney cells, monkey kidney cells or bovine kidney cells (Awang and Russell 1990).

**Serology**

The haemagglutination inhibition test can be used to identify all avian paramyxoviruses except APMV-5 (Alexander and Senne 2008b). Cross reactions between serotypes are possible, particularly between APMV-3 and NDV (Alexander and Senne 2008b; Warke et al. 2008a).

**Transmission via eggs**

There is no evidence that APMV-3 can be transmitted vertically. It is possible that APMV-3 may be present in faeces and be a contaminant on the eggshell.

**Biosecurity significance**

APMV-3 is not an OIE-listed disease agent.

APMV-3 infection is not notifiable in any state or territory of Australia, and is not subject to official controls. APMV-3 is not included in the Emergency Animal Disease Response Agreement.

**Conclusion**

- There are reports of clinical respiratory disease in younger turkeys associated with APMV-3 infections (Redmann et al. 1991).
- The prevalence of APMV-3 in the countries approved to export hatching eggs to Australia is unknown (Warke et al. 2008a).
- There is no evidence that APMV-3 is transmitted via eggs. It is possible that APMV-3 may be present in faeces and be a contaminant on the eggshell.
- There is a low rate of spread between birds and between flocks (Alexander et al. 1983; Le Gros 1986). The mechanism of spread of APMV-3 between flocks is unknown (Alexander and Senne 2008a).
- APMV-3 is not a robust organism and is inactivated by a number of common disinfectants (Quinn and Markey 2001). The current import conditions require the surface of imported eggs to be disinfected twice—at egg collection and at arrival at the quarantine station. The use of an effective disinfectant is likely to inactivate any APMV-3 contaminating the outside of the shell.

2.2.2 **Risk assessment**

Based on the above information, the department concluded that further risk assessment was required.
Hazard identification

APMV-3 is recognised as a hazard as defined by the Code as evidenced by:

- it is appropriate to the species being imported, in this case turkeys and chickens. Although APMV-3 is not a natural infection of chickens, they may be infected experimentally and could introduce APMV-3 into Australia with subsequent spread to the Australian turkey flock
- it is present in some exporting countries and is not known to be present in Australia
- based on the conclusions above, APMV-3 is capable of producing adverse consequences in chickens and turkeys
- APMV-3 is also capable of producing adverse consequences in passerine and psittacine birds.

Risk assessment

Entry assessment

The conclusions:

- the prevalence of APMV-3 in exporting countries is likely to be less than previously assumed but the agent is present in a number of countries exporting hatching eggs to Australia
- although there is no evidence for vertical transmission, the agent may be present on the eggshell
- the virus is relatively susceptible to common disinfectants
- given the usual lack of clinical signs in adult flocks, the general health requirements for donor flocks in the Conditions does not reduce the risk.

Therefore, the overall assessment is that the likelihood of entry of APMV-3 into Australia via the importation of hatching eggs would be **low**.

Exposure assessment

After completion of quarantine, the birds hatched from imported eggs are typically moved immediately to poultry breeding complexes. This means they are introduced directly into the Australian poultry flock and so the likelihood of exposure of local poultry should APMV-3 enter via imported hatching eggs is assessed as being **high**.

Consequence assessment

Under the scenario of introduction through imported hatching eggs APMV-3 would be introduced into the Australian poultry flock at the very highest level—high biosecurity primary breeding stocks. From those flocks there is the possibility of spread to high and medium biosecurity flocks across the entire country. Although the consequences of introduction of a disease are independent of the route of entry, a previous assessment undertaken on this agent in the *Generic import risk analysis report for chicken meat 2008* (Chicken meat IRA) (Biosecurity Australia 2008) did not assess this scenario. That analysis concludes that the highest level of the poultry flock than can be exposed to the agent in imported chicken meat was medium biosecurity flocks, there would not be
spread to flocks of higher status and higher biosecurity and therefore spread and impact would be limited.

The consequences are assessed using the methodology described in the Chicken meat IRA.

- The direct impacts on poultry are assessed as being minor at a national level.
- The direct impacts on the environment, in particular the potential effects on native birds, are assessed as being minor at a state/territory level.
- The indirect effects on poultry, either the commercial decision to eradicate affected flocks or on-going vaccination, are assessed as being unlikely to be discernible at a national level.
- All other indirect effects are assessed as being negligible.

As at least one of the impacts, the direct effect on poultry, is assessed as minor at a national level, the overall impact is assessed as moderate.

Risk estimation

As the likelihood of entry and exposure is estimated as low (by multiplication—see the methodology described in the Chicken meat IRA) and the likely consequences are assessed as moderate then the risk assessment of this agent is low. This exceeds Australia’s ALOP and risk management is required.

2.2.3 Risk management

- Flocks producing eggs for export to Australia (source flocks) are to be certified as free from APMV-3 during the period of 90 days prior to egg collection.
- Eggs are to undergo fumigation or disinfection after collection and again after arrival in Australia.
- Source flocks are to be serologically tested for APMV-3 with in 21 days before the first day of collection of eggs. It is recognised that cross-reactions between APMV-3 and NDV will occur.
  - Unvaccinated flocks—a random sample of sufficient size to give 99% confidence of detecting the agent if there is 5% prevalence in the source flocks is tested. Positive serology may indicate active infection of APMV-3 within the source flocks.
  - Vaccinated flocks—a random sample of 100 individually identified birds is to be tested with individual titres to be recorded for each bird sampled. The test is repeated on these same birds not less than 14 days after the collection of the last eggs for the consignment. A rise in titre may indicate an active infection of APMV-3 within the source flocks.

OR

Validated PCR test. A random sample of sufficient size to give 99% confidence of detecting the agent if there is 5% prevalence in the source flocks is tested within 21 days before the first day of collection of eggs and not less than 14 days after the collection of the last eggs for the consignment.
• Cloacal swabs are taken from a sample of the PAQ flock sufficient to give a 99% confidence of detecting 5% disease prevalence in the flock and the samples are tested for haemagglutinating agents. This testing will detect the presence of APMV-3.

Actions to be taken in response to the detection of APMV-3

If the results of any testing or investigation indicate the presence of APMV-3 then, at the discretion of the department, further investigations and additional testing may be performed to determine the cause of the positive result. It is recognised that serology for APMV-3 may be complicated by cross-reactions with other avian paramyxoviruses and results should be assessed on a case by case basis. Any additional tests or investigations will be at the importer’s expense.

As with any of the diseases listed in the Conditions as being of quarantine concern, the quarantine flock may be destroyed if it is confirmed that it is infected with APMV-3.

References


