REVIEW OF THE IMPORT POLICY FOR SPF EGGS

EXECUTIVE SUMMARY

Three Australian vaccine manufacturers have requested that Biosecurity Australia remove the contingency clause from the current policy on the importation of specific pathogen free (SPF) eggs for vaccine production and to allow the use of non-Australian origin SPF eggs in live avian vaccines. The manufacturers' main concern is continuity of supply of SPF eggs, which are critical to production of many avian vaccines. Their requests raise a number of complex issues that have potential to impact on various industry and disease control programs.

SPF eggs are essential in the production of many veterinary vaccines and some human viral vaccines. They are also used for quarantine monitoring and sentinel programs, the diagnosis of some diseases and biomedical research and development. Recent shortfalls in the Australian production of SPF eggs have raised further concern that the current contingency policy is unable to meet the needs of essential human and animal disease control programs should shortfalls continue. However, issues such as domestic production of SPF eggs are outside the responsibility of government and need to be resolved by industry.

Problems do occur with the use of SPF eggs as evidenced recently by disease breakdowns in SPF flocks in the USA, Germany and Mexico and lead, in some cases, to contaminated vaccines. A contaminated vaccine has the potential to cause multi-focal outbreaks of disease associated with the contaminant, with the disease becoming established nationally very quickly.

Compliance with the European Pharmacopoeia requirements for SPF flocks is sufficient to address Australian animal quarantine concerns with imported SPF eggs for *in vitro* laboratory use, for use in human vaccines or inactivated veterinary vaccines subject to appropriate quarantine controls on importation, transport, storage, use and disposal of the eggs and associated waste. In addition, human health concerns with the use of SPF eggs in human vaccines and therapeutics would be addressed by the Therapeutic Goods Administration of the Australian Government Department of Health and Ageing.

Australian animal quarantine concerns with imported SPF eggs for *in vitro* laboratory use, for use in human vaccines or inactivated veterinary vaccines can be addressed by ensuring the source flock meets an appropriate standard of animal health (eg European Pharmacopoeia) and that there are appropriate quarantine controls on transport, storage, use and disposal of the eggs and associated waste. In addition, human health concerns with the use of SPF eggs in human vaccines and therapeutics would be addressed by the Therapeutic Goods Administration of the Australian Government Department of Health and Ageing.

Live avian vaccines are considered to be the highest risk for the use of SPF eggs due to a history of contamination, a lack of any significant extraneous agent inactivation

step and target species. The history of contamination of live avian vaccines suggests that the level of controls currently applied internationally to live avian vaccines may not be sufficient to address Australia's quarantine concerns. Biosecurity Australia therefore considers that controls, above and beyond those currently applied by these international standards, are required for live avian vaccines produced on SPF eggs of non-Australian origin. These additional controls could be applied to either the source SPF flock (eg increased sampling and testing) or the bulk/finished live avian vaccine (eg more sensitive extraneous infectious agent testing). A further review would be required in the area of appropriate, highly sensitive extraneous infectious agent testing on live avian vaccines. Until such a review is completed, the option of using more sensitive testing on the final vaccine will only be available following case by case assessment. This assessment would require detailed justification, based on test sensitivity in detecting very low titres of extraneous agent. Applications would also be subject to individual public consultation.

A shortage of SPF eggs could have a critical impact on human and animal health within Australia, necessitating importation. However, the use of SPF eggs of non-Australian origin, especially in the production of live avian vaccines, is considered a high quarantine risk. It is therefore recommended that the use of these in live avian vaccines be contingent on demonstration of a critical national need for the vaccine. It is recommended that this contingency clause be removed in 12 months, provided issues such as additional highly sensitive extraneous agent testing are resolved.

It should be noted that many of the problems with SPF flock breakdowns and vaccine contamination are due to pathogens such as chicken anemia virus and avian leucosis virus, which are endemic in Australia. However, in line with our international trading obligations, controls may not be imposed on endemic pathogens additional to those imposed by the Australian Pesticides and Veterinary Medicines Authority (APVMA) on Australian SPF flocks and veterinary vaccines produced in Australia on Australian SPF eggs.

Based on this review of the import policy on SPF eggs, Biosecurity Australia has developed a draft "Quarantine policy for the importation and/or use of fertile specific pathogen free (SPF) eggs (*Gallus gallus*) of non-Australian origin". This draft policy and its associated condition sets is available for public consultation and is being released concurrently with this review.

INTRODUCTION

An import policy for the importation of specific pathogen free (SPF) eggs for vaccine production was developed in June 1998. The policy is only to be used on a 'contingency basis', that is, in the event of a failure in domestic SPF egg supply with subsequent potential reduction of vaccine availability for disease control purposes. Under the 1998 policy, the use of imported SPF eggs in live avian vaccines is not permitted.

Biosecurity Australia has received requests from 3 vaccine manufacturers in Australia to remove the contingency clause from the current policy on the importation of SPF eggs for vaccine production and permit the use of SPF eggs of non-Australian origin in live avian vaccines. The manufacturers' main concern is continuity of supply. Their requests raise a number of complex issues that have potential to impact on various industry and disease control programs.

In February 2002, Biosecurity Australia coordinated a meeting with key industry and government stakeholders. As an outcome of this meeting, Biosecurity Australia undertook to review its import requirements for SPF eggs. However, it was also evident that many of the issues involving availability of SPF eggs were outside the responsibility of government and needed to be resolved by industry.

The SPF egg industry in Australia is small due to limited domestic demand. There is now only one SPF egg producer in Australia (SPAFAS) making the impact of a potential breakdown in production very significant. There are few major users of SPF eggs and significant fluctuations in demand. The SPF egg producer requires considerable prior notice in order for production to meet demand. Globalisation of the vaccine industry and SPF egg production has led to increased demand for greater flexibility in sourcing of the eggs.

SPF eggs are essential for:
- Veterinary vaccines, especially avian vaccines
- Some human viral vaccines eg Q-fever vaccine
- Quarantine monitoring and quarantine sentinel programs
- Disease diagnosis eg virus isolation
- Biomedical research and development

Shortfalls in the Australian production of SPF eggs in 2003 have further raised concern that the 1998 contingency policy is unable to meet the needs of essential human and animal disease control programs. Biosecurity Australia considers it desirable that all Australian users continue to have access to SPF eggs.

SPF eggs are less likely to be infected or contaminated with pathogens than non-SPF embryonated eggs. Despite this, problems can and do occur. For example, in 2003, there were biosecurity breakdowns in SPF flocks in Germany, Mexico and USA with reovirus, avian leucosis virus and avian adenovirus. As a result, there have been reports of vaccines contaminated with avian reovirus in Europe and avian leucosis virus in USA. In addition, there are the ongoing problems in the USA with chicken anemia virus in many SPF flocks. The potential for vaccines to be contaminated with

extraneous infectious agents is well documented. A summary of reports of vaccine contamination is available from Biosecurity Australia on request. A contaminated vaccine has the potential to spread a pathogen with disease outbreaks quickly becoming established nationally before the source is identified. However, not all SPF eggs are used for vaccine production as a small proportion are used for lower risk purposes such as *in vitro* laboratory use.

ASSESSMENT OF RISKS ASSOCIATED WITH END USE

In Vitro Laboratory Use

SPF eggs are often used for virus isolation, quarantine surveillance, quality control of vaccines and research and development in the biomedical and biotechnology fields. This work is conducted in laboratories and usually does not involve exposure of animals. As a general principle, all biological waste generated in laboratories is autoclaved, incinerated or otherwise disposed of safely.

While there are inherent quarantine risks associated with importation and use of the imported eggs, these risks are significantly reduced if the eggs and/or their derivatives:
  a. are from SPF flocks that meet high standards of animal health; and
  b. are not exposed to susceptible species without additional risk assessment; and
  c. do not leave the laboratory without AQIS approval and are disposed of safely; and
  d. are restricted to laboratories which are an AQIS Quarantine Approved Premises (QAP) for the purposes of handling imported SPF eggs to ensure compliance with the above control measures.

Vaccine Production and other *In Vivo* Uses

There are inherent risks associated with vaccines and substrates, including embryonated eggs, used in vaccine production. A review of the history of vaccines contaminated with extraneous infectious agents is attached. A contaminated vaccine could rapidly spread a pathogen nationally, making eradication very difficult.

SPF flocks and eggs used to produce vaccines for use within Australia are expected to meet the requirements specified in the current European Pharmacopoeia (EP). AQIS requires, under the *Quarantine Act 1908*, that veterinary vaccines be demonstrated to be free of pathogens of quarantine concern to Australia. The Australian Pesticides and Veterinary Medicines Authority (APVMA) requires that veterinary vaccines be demonstrated free of all infectious contaminants. To avoid duplication, AQIS assessment of imported veterinary vaccines covers both quarantine and APVMA requirements in relation to freedom from all extraneous infectious agents.

End-product testing using embryonated egg and chick inoculation is very effective in detecting extraneous agents, provided there is sufficient agent present in the amount of vaccine inoculated into each egg or chick to initiate infection. However, if a disease has escaped detection in the SPF flock, the subsequent titre in contaminated eggs may be very low. In this case, the titre of the extraneous agent in the final vaccine may also

be very low yet the contaminated vaccine could still result in a substantial number of infections if administered to several thousand birds.

The European Pharmacopoeia extraneous agent testing, using both embryonated eggs (EP 2.6.3) and chicks (EP 2.6.6), is arguably more sensitive than the equivalent standards required by the US Code of Federal Regulations (9CFR113.37 and 9CFR113.36 respectively). Provided there is at least one EID_{50} or CID_{50} of the extraneous agent per dose of vaccine, the likelihood of detecting the contaminant by either of these methods is almost certain. At much lower levels of contamination, detection becomes less likely.

The intention of inactivation of vaccines is to remove the organism's infectiousness but not its ability to stimulate an immune response. Assessment of the inactivant (eg formalin, etc) is based on its effectiveness against the vaccine organism and not against potential extraneous agents. However, the use of most inactivants will result in at least some titre reduction of any infectious agent. Where the titre of the extraneous agent in the final vaccine is already extremely low, the use of an inactivant may be enough to render the product safe. As a result, there are fewer reports of contaminated inactivated vaccines than live vaccines.

There has been recent reports of chicken anaemia virus (CAV), avian adenovirus, avian leucosis virus and avian reovirus in SPF flocks and of avian vaccines subsequently contaminated with avian reovirus and leucosis viruses. These reports have highlighted the potential risk associated with the use of SPF eggs in vaccine production and short-comings of current surveillance and testing of both SPF flocks and live avian vaccines.

Therefore, Biosecurity Australia is concerned that the controls and testing regimes typically applied to SPF flocks in accordance with internationally recognised standards, such as the European Pharmacopoeia, may not be sufficient to address Australia's quarantine concerns, especially with live avian vaccines. To address this, it may be necessary to apply the following measures in respect to all exotic viral pathogens that are also potential contaminants:

- additional sampling and testing of the SPF flock prior to use of eggs in the production of the live avian vaccine; or
- more sensitive extraneous agent detection tests on final/bulk live avian vaccines.

Responsibility for assessment and registration of domestic vaccines rests with APVMA. AQIS cannot apply controls on CAV, avian leucosis and other endemic pathogens above that applied by APVMA to domestic vaccines. However, vaccine manufacturers and the Australian poultry industry should be aware of the above concerns in relation to SPF eggs and consider the use of highly sensitive detection tests or increased flock testing, especially for CAV and avian leucosis.

EXOTIC DISEASE RISK ASSESSMENTS

The following are disease agents which are either exotic to Australia, or for which there are exotic strains. These disease agents were identified as significant hazards in the Technical Issues Paper of the Egg and Egg Products Import Risk Analysis (IRA).

Avian Influenza

Most avian influenza viruses (AIV) are of low or mild pathogenicity (LP or MP), producing either subclinical disease or mild respiratory or reproductive disease in domestic and wild birds. Highly pathogenic (HP) avian influenza (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 recognized 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.

Low pathogenicity avian influenza viruses are distributed worldwide in many species of domestic and wild birds, including chickens, turkeys, domestic and wild waterfowl and game birds, passerines, psittacines, raptors and ratites (Easterday; Hinshaw, and Halvorson 1997). Wild birds, particularly wild aquatic birds such as ducks, gulls and shorebirds, are believed to provide a reservoir of avian influenza viruses, with asymptomatic enteric infections leading to faecal shedding of virus.

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 Holland, Belgium and Germany (Shane 2003). Outbreaks of HPAI occurred in Australia in 1976, 1985, 1992, 1995 and 1997 (Swayne and Suarez 2000). Currently, there are outbreaks of H5N1 HPAI in Eastern Asia (http://www.promedmail.org).

Avian influenza virus can be present within, 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 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 ℃.

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). It is possible that eggs from viraemic hens could be distributed before a diagnosis is made.

Detection methods

The European Pharmacopoeia specifies the following test protocols:

- ELISA testing of the SPF flock (5% of flock tested monthly), and

- agar gel precipitation (AGP) of the final live avian vaccine, on ten 2-week old chicks following inoculation with 100 doses of live avian vaccine intramuscular and 10 doses intraocular, repeat inoculations 2 weeks later, and testing of birds at 5 weeks after first inoculation.

Various molecular detection methods are available for avian influenza virus (refer Appendix 1).

Risk management

While HPAI is likely to spread very quickly through a naïve flock (ie the SPF flock) and be readily detected, LPAI may not. A contaminated vaccine could infect a large number of flocks in a very short time leading to a multifocal outbreak. There may also be a significant risk of reassortment of virus if a avian influenza virus is introduced into poultry populations via contaminated vaccine. The national impact associated with a contaminated vaccine could therefore be significant depending on the pathogenicity of the virus. Therefore, more stringent risk management measures are required. Additional sampling and testing of the SPF flock or much more sensitive detection tests on the final vaccine than required by standards such as the European Pharmacopoeia are recommended for AIV where the SPF eggs are used in live avian vaccine production.

Avian adenoviruses

Group 1 Fowl Adenovirus (FAdV), serotype 4 is the causative agent of the disease variously known as hydropericardium syndrome (HPS), Angara disease, hydropericardium-hepatitis syndrome, infectious hydropericardium and inclusion body hepatitis/hydropericardium syndrome. The disease was first reported in the Angara Goth region of Pakistan in 1988, and resulted in over 100 million deaths in meat chickens, with mortality rates up to 75% in individual flocks (Toro et al 1999). Hydropericardium syndrome has since been reported in other countries in Asia, the Middle East, Russia, Central and South America (Ganesh and Raghavan 2000;McFerran and Smyth 2000), often in association with other viruses such as IBDV, Marek's Disease or Chicken Anaemia Virus. The disease has not been reported in Australia or New Zealand. Vertical transmission of Group 1 avian adenoviruses occurs. Vertical transmission of HPS has been demonstrated in experimentally-infected layer breeders.

Avian adenovirus splenomegaly (AAS) is an avian adenovirus group 2 viral disease of chickens related to haemorrhagic enteritis of turkeys and marble spleen disease of pheasants. Although haemorrhagic enteritis and marble spleen disease have been reported in Australia (Tham and Thies 1988), AAS of chickens has not.

Unlike group 1 avian adenoviruses, no evidence for egg transmission of group 2 avian adenovirus has been found (McFerran and Smyth 2000). Transmission of AAS is via the faecal-oral route, so there is potential for surface contamination of eggs.

Detection methods

Specific tests for avian adenoviruses as per the European Pharmacopoeia are ELISA on the SPF flocks (5% of flock tested monthly) and agar gel precipitation (AGP) on ten 2-week old chicks following inoculation with 100 doses of live avian vaccine

intramuscular and 10 doses intraocular, repeat inoculations 2 weeks later, and test birds at 5 weeks after first inoculation.

PCR can be used to detect FAdV serotypes 1-12 and a specific PCR is available for serotype 4. Refer to Appendix 1.

Risk management

As with any extraneous agent, a contaminated vaccine could infect a large number of flocks in a very short time leading to a multifocal outbreak with a potential national impact. Therefore, based on egg transmission, history of SPF flock breakdowns with avian adenovirus, the severity of several adenovirus serotypes, risk management measures are justified for avian adenoviruses in Group 1. Additional sampling and testing of the SPF flock or much more sensitive detection tests on the final vaccine than required by standards such as the European Pharmacopoeia are recommended for avian adenovirus type 1 where the SPF eggs are used in live avian vaccine production.

As there is no evidence of egg transmission, the current European Pharmacopoeia testing requirements are considered to be adequate for avian adenoviruses in Group 2.

Avian Pneumovirus (Turkey Rhinotracheitis)

Avian pneumovirus (APV) is a pneumovirus, the type species for the genus *Metapneumovirus*. It is a significant respiratory pathogen in both turkey and chicken flocks, causing serious economic losses in birds of any age. The disease caused by APV in turkeys is also known as turkey rhinotracheitis or turkey coryza. APV also infects chickens and is associated with swollen head syndrome in meat chickens and breeders (Cook 2000). Australia is reported free from APV (Bell and Alexander 1990).

APV has been demonstrated in the reproductive tract of experimentally infected laying turkeys. The virus may be shed in faeces and respiratory secretions and thus contaminate the shell of eggs.

Detection methods

The European Pharmacopoeia recommends that the SPF flocks are tested using an ELISA, with 5% of the flock being tested monthly. An ELISA based on the matrix (M) protein of avian pneumovirus (APV) is claimed to be a highly sensitive and specific test for detecting antibodies to APV with a sensitivity of 97% compared with only 52% for a routine ELISA (Gulati et al 2000).

Various molecular detection methods are available for avian pneumovirus (refer Appendix 1).

Risk management

Based on the possible presence of APV in the reproductive tract, the severity of the disease, low sensitivity of the routine ELISA test and the potential national impact of multifocal outbreaks, risk management measures are justified for avian pneumovirus. Where the SPF eggs are used in live avian vaccine production, additional sampling and testing of the SPF flock or the use of much more sensitive detection tests on the

final vaccine than required by standards such as the European Pharmacopoeia are therefore recommended for avian pneumovirus.

Infectious Bronchitis

Infectious bronchitis is an acute, contagious viral disease of chickens and pheasants, which results in high morbidity and variable mortality in affected flocks. The aetiological agent is a coronavirus. The disease can be manifested as a respiratory syndrome, characterised by coughing, sneezing and tracheal rales; a nephritic syndrome, in which the kidneys are the primary target organs, or as a reproductive syndrome characterised by a drop in egg production and quality. IBV strains tend to differ between geographic regions, and emergence of variant strains is relatively common. Therefore, although IBV is endemic in Australia, if some exotic strains were introduced, currently available vaccination programs would be inadequate to prevent disease (Ignjatovic and Sapats 2000).

Some strains of infectious bronchitis show greater tropism for the reproductive tract than others (Crinion and Hofstad 1972). Those strains that do cause pathology of the reproductive tract cause alterations in egg production, shell quality and albumen quality (Sevoian and Levine 1957). Resumption of faecal shedding of virus has been documented at point of lay in hens experimentally infected at one-day-old with infectious bronchitis virus (Jones and Ambali 1987). Therefore, the surface of eggs could be contaminated with virus, long after disappearance of clinical signs in the flock.

The role of vertical transmission of infectious bronchitis virus has not been firmly established (Ignjatovic and Sapats 2000). Nevertheless, virus has been demonstrated in the embryonated eggs of naturally- (McFerran et al 1971) and experimentally-infected hens (Cook 1971). Virus was also isolated from the yolk of eggs laid by experimentally infected hens, from 2-43 days post-inoculation (Fabricant and Levine 1951).

Detection methods

The European Pharmacopoeia specifies the following test protocols:

- ELISA testing of the SPF flock (5% of flock tested monthly), and

- agar gel precipitation (AGP) or haemagglutination inhibition of the final live avian vaccine, on ten 2-week old chicks following inoculation with 100 doses of live avian vaccine intramuscular and 10 doses intraocular, repeat inoculations 2 weeks later, and testing of birds at 5 weeks after first inoculation.

For surveillance purposes, an ELISA may be the most appropriate antibody detection method for detecting antibodies to IBV (Ignjatovic and Sapats 2000). The sensitivity of antibody capture ELISA varies from 83% to 100% depending on the time between after challenge and sampling. Following experimental challenge, AGP only had a sensitivity of 40% and the sensitivity of both HI and VN varied widely (Wit JJ de et al 1992;Wit JJ de et al 1998;Wit JJ de et al 1997).

Various molecular detection methods are available for infectious bronchitis virus (refer Appendix 1).

Risk management

This review could not find any references to vaccines contaminated with IBV or other coronaviruses. However, based on probability that IBV is egg transmitted and the potential national impact of a multifocal outbreak of an exotic strain of the virus, risk management measures are required. While the use of ELISA on the SPF source flock, in accordance with current European Pharmacopoeia requirements, provides considerable confidence, the sensitivity of AGP or HI on the final vaccine may not. Additional sampling and testing of the SPF flock or more sensitive detection tests of the final vaccine than required by the European Pharmacopoeia are recommended for infectious bronchitis where the SPF eggs are used in live avian vaccine production.

Avian Reovirus

Avian reoviruses are the cause of infectious viral arthritis/tenosynovitis of poultry. Although reoviruses have also been associated with a number of other poultry disease conditions including malabsorption syndrome, runting/stunting syndrome, diarrhoea, respiratory disease and sudden death, in many cases a causal association is not proven. There are exotic strains of avian reovirus causing arthritis/tenosynovitis.

Viral arthritis/tenosynovitis is predominantly a disease of meat chickens; however, it has also been reported in layer flocks in the USA and UK (Schwartz; Gentry, and Rothenbacher 1976;Kibenge and Wilcox 1983). In Australia, tenosynovitis has been observed only in meat-type chickens (Kibenge and Wilcox 1983).

Reovirus is readily transmitted via the embryonated egg where it multiplies on the chorioallantoic membrane of embryonated chicken eggs. (Deshmukh and Pomeroy 1969).

Preparations of Marek's vaccine contaminated with live reovirus have been reported (Simmons et al 1972). There has also been an unverified report in 2003 of a vaccine manufacturer detecting avian reovirus in its vaccines after 2 German flocks became infected with the virus (Pers.Com. SPAFAS Australia 2003).

Detection methods

The European Pharmacopoeia specifies the following test protocols:

- fluorescent antibody testing of the SPF flock (5% of flock tested monthly), and

- agar gel precipitation (AGP) of the final live avian vaccine, on ten 2-week old chicks following inoculation with 100 doses of live avian vaccine intramuscular and 10 doses intraocular, repeat inoculations 2 weeks later, and testing of birds at 5 weeks after first inoculation.

Various molecular detection methods are available for infectious bronchitis virus (refer Appendix 1).

Risk management

Because avian reovirus is egg transmitted, a history of infected flocks and contaminated vaccines and the potential national impact of a multifocal outbreak of an exotic strain of the virus, risk management measures are required. Additional sampling and testing of the SPF flock or more sensitive detection tests of the final vaccine than required by the European Pharmacopoeia are recommended for avian reovirus where the SPF eggs are used in live avian vaccine production.

Newcastle Disease

Strains of Newcastle disease virus (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), lentogenic (mildly virulent) and avirulent (Alexander 1997).

The natural hosts of NDV 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). NDV is an OIE list A disease agent and is a notifiable disease in all Australian states and territories. NDV 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).

Outbreaks of virulent Newcastle disease occurred in Australia in 1930 and 1932, 1998, 1999 and 2000 (Westbury 2001), and 2002. Avirulent and lentogenic strains of ND virus have been isolated in a number of countries (McNulty et al 1988;Durham et al 1980;Westbury HA 1981). Avirulent strains of ND virus are endemic in poultry flocks in Australia (Westbury HA 1981), but the emergence of strains of low virulence associated with mild disease (termed late respiratory syndrome) in the 1990s (Hooper et al 1999) and, more recently, virulent strains, suggests that there has been a gradual evolution in Australian strains of NDV (Westbury 2001).

NDV 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 to lay and infected embryos die (Beard and Hanson 1984). Recent studies have shown that 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).

Three live avian vaccines produced by one company and used in Denmark in 1996/97 were contaminated with APMV-1 viruses of low virulence for chickens (Jorgensen et al 2000). A recent study also found that a Newcastle disease vaccine contained a V4

strain instead of the intended VGGA strain. The study also found several batches of monovalent and combined La Sota NDV vaccine to be contaminated with strain B-1 (Farsang et al 2003).

Detection methods

The European Pharmacopoeia specifies the following test protocols:

- haemagglutination inhibition testing of the SPF flock (5% of flock tested monthly), and

- haemagglutination inhibition of the final live avian vaccine, on ten 2-week old chicks following inoculation with 100 doses of live avian vaccine intramuscular and 10 doses intraocular, repeat inoculations 2 weeks later, and testing of birds at 5 weeks after first inoculation.

Various molecular detection methods are available for Newcastle disease virus (refer Appendix 1).

Risk management

While velogenic NDV is likely to spread very quickly through a naïve flock (ie the SPF flock) and be readily detected, mesogenic and lentogenic strains may not. Introduction of any new strain of NDV could therefore result in the further development and/or evolution of NDV within Australia. The national impact associated with a contaminated vaccine could therefore be significant depending on the pathogenicity of the virus in Australian birds. Risk management measures are therefore required.

Additional sampling and testing of the SPF flock or much more highly sensitive detection tests above that required by the European Pharmacopoeia is recommended for Newcastle disease where the SPF eggs are used in live avian vaccine production.

Because NDV is egg transmitted, a history of infected flocks and contaminated vaccines and the potentially high national impact of a multifocal outbreak, risk management measures are required. Additional sampling and testing of the SPF flock or more sensitive detection tests of the final vaccine than required by the European Pharmacopoeia are recommended for Newcastle disease virus where the SPF eggs are used for live avian vaccine production.

Other Avian Paramyxoviruses

Avian paramyxovirus-2 (APMV-2)

Avian paramyxovirus-2 (APMV-2) infection in poultry has been associated with inapparent or mild respiratory disease, except where infection is complicated by the presence of other pathogens. Although infection has been reported in chickens, turkeys, and caged passerines and psittacines, the primary natural host appears to be small passerine birds (Alexander 1993).

APMV-2 appears to have a worldwide distribution in various hosts. In poultry, APMV-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). In countries where monitoring and surveillance is carried out, APMV-2 has been isolated from wild passerine birds, and from captive caged passerines and psittacines. APMV-2 has not been reported in poultry in Australia or New Zealand.

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

Avian paramyxovirus-3 (APMV-3)

Avian paramyxovirus-3 (APMV-3) infection causes respiratory signs and decreased egg production in turkey flocks. Although turkeys are considered to be the primary natural host of APMV-3, experimental studies have shown that chickens are susceptible to infection (Alexander 1997) and there is one report of isolation of APMV-3 from a flock of chickens with respiratory disease (Shihmanter et al 2000). APMV-3 has also been isolated from captive dead or dying psittacine and passerine birds held in quarantine (Alexander 1997). Isolates show considerable diversity and two antigenically distinguishable groups are recognised. The first has been isolated only from turkeys, and the second from captive, caged psittacine and passerine birds, and recently from a chicken flock in Israel (Shihmanter et al 2000).

Most reports of natural APMV-3 infections in domestic poultry are from turkeys in Western Europe, North America and Israel. APMV-3 has not been reported in poultry in Australia or New Zealand.

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

Risk management of APMV-2 and APMV-3

APMV-2 and APMV-3 are not routinely tested under European Pharmacopoeia requirements. Use of a contaminated vaccine could result in multifocal outbreaks. Where the SPF eggs are to be used in live avian vaccine production, it is recommended that either:

- the flock be tested free of the diseases by routine monthly testing over the preceding 12 month period at the European Pharmacopoeia sampling rate; or

- the SPF flock be tested free of the disease with the 21 day period prior to egg collection at a sample rate sufficient to detect the disease with 99% confidence if it occurs at a prevalence of 0.5% (taking into account test sensitivity); or

- the bulk or final vaccine be tested for the pathogen using a highly sensitive detection test.

Infectious Bursal Disease

Infectious bursal disease (IBD) is an acute, contagious viral infection, which causes immunosuppression in young chicks, and disease and mortality in 3-6 week old chickens (Lukert and Saif 1997;van den Berg et al 2000). IBD viruses can be classified according to virulence, as attenuated (vaccine strains), classical virulent, variant and very virulent (vvIBDV, sometimes known as hypervirulent) (van den Berg

et al 2000). Classical and Australian variant strains exist in Australia, which can be genetically differentiated from overseas classical, variant strains and very virulent strains (Sapats and Ignjatovic 2000;Ignjatovic and Sapats 2002). Infectious bursal disease is an OIE list B disease.

There is no evidence that IBDV 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 IBDV, and would be unlikely to shed virus in the faeces. In an unpublished experimental trial, IBDV was not isolated from the yolk, albumen or shell of eggs laid by non-vaccinated 24-week old chickens challenged with vvIBDV, despite the presence of virus in cloacal swabs for 2-3 days after inoculation (unpublished report, AAHL).

Risk management

The European Pharmacopoeia specifies the following test protocols:

- immunodiffusion testing of the SPF flock (5% of flock tested monthly) against each serotype present in the country of origin, and

- agar gel precipitation (AGP) of the final live avian vaccine, on ten 2-week old chicks following inoculation with 100 doses of live avian vaccine intramuscular and 10 doses intraocular, repeat inoculations 2 weeks later, and testing of birds at 5 weeks after first inoculation.

As there is no evidence of direct egg transmission and no reports of vaccines contaminated with IBDV, the routine sampling and testing in accordance with European Pharmacopoeia requirements should be sufficient to address Australian quarantine concerns with IBDV.

Salmonella spp

<u>*Salmonella* Arizonae</u>

Salmonella Arizonae is used to designate a group of bacteria comprising some 415 different antigenic types (Davos 2001). Arizonosis in turkeys is an acute systemic disease that may cause significant economic losses due to reduced egg production and hatchability, and morbidity and mortality in poults. 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). Historically, isolates from chickens and turkeys in the USA and United Kingdom were of two serovars, $18:Z_4,Z_{32}$ (original designation 7a,7b:1,7,8:-) and $18:Z_4,Z_{23}$ (original designation 7a,7b:1,2,6:-) (Hall and Rowe 1992;Silva et al 1980). Reports suggest that a change has occurred in the relative proportion of the two serovars, and that isolation of $18:Z_4,Z_{23}$ is now rare (Shivaprasad et al 1997). Serovar $18:Z_4,Z_{32}$ has not been isolated in Australia (Davos 2001).

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. This has been shown to occur in around 5% of chicken eggs (Williams and Dillard 1968a).

Pullorum and *fowl typhoid*

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 diseases are similar in terms of epidemiology and management (Shivaprasad 1997;Shivaprasad 2000;Wray and Davies 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. These two diseases have been eradicated from Australian commercial flocks (Anonymous 1998). 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).

S. Pullorum is vertically transmitted, with organisms localising in the ovule or contaminating the ovum following ovulation. *S*. Pullorum was detected in eggs laid by commercial layer hens that had been experimentally infected at 4-5 days of age (Berchieri et al 2001) and in another study at one week of age (Wigley et al 2001;Pinheiro; de Oliveira, and Berchieri 2001).

Although vertical transmission of *S*. Gallinarum has been 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.

S. Enteritidis and *S. Typhimurium*

S. Enteritidis and *S*. Typhimurium are typically non-host specific 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 1997).

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. In 1994, *S*. Enteritidis PT 4 was isolated from a commercial layer flock in Australia. However the isolates were thought to be the result of laboratory contamination since isolation could not be repeated on resampling of the same shed (Davos 2002).

Over 270 phage types of *S*. Typhimurium are recognised, of which definitive type (DT104) is probably the most important. Multiple antibiotic resistant strains of *S*. Typhimurium DT 104 have not been isolated from poultry flocks in Australia (Anonymous 1999). 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; Nicholls, and Barton 1999).

Intact shell eggs have been implicated as the major vehicle of transmission of *S.* Enteritidis in a number of countries (Cox 1995). Although experimental studies have shown that both *S.* Enteritidis and *S.* Typhimurium are able to colonise the reproductive tract and eggs at equivalent rates (Keller et al 1997), transovarian contamination of commercially produced eggs with *S.* Typhimurium is rare (Keller et al 1997).

Detection methods for Salmonella spp

Specific tests for *Salmonella spp* as per the European Pharmacopoeia for the SPF flocks (5% of flock tested monthly) are agglutination for *Salmonella pullorum* and culture of faecal samples every 4 weeks for *Salmonella spp*. Agglutination is also used to test live avian vaccines for *Salmonella pullorum* using ten 2-week old chicks following inoculation with 100 doses of live avian vaccine intramuscular and 10 doses intraocular, repeat inoculations 2 weeks later, and test birds at 5 weeks after first inoculation. Appropriate salmonella culture and detection methods are detailed in Section 2.6.13 of the European Pharmacopoeia and by Australian Standard AS1766.2.5.

Risk management for Salmonella spp

Contamination of eggs with the above *Salmonella spp* is possible and represents a significant risk of contaminating vaccines. Current risk management measures are considered to provide adequate quarantine confidence. Current measures to prevent contamination include the routine surveillance and monitoring for Pullorum, fowl typhoid and other Salmonella species in accordance with the European Pharmacopoeia and also testing of the bulk or final vaccine for Salmonella contamination in accordance with current Australian vaccine import policies.

Haemophilus paragallinarum (Infectious Coryza)

Infectious coryza is an acute to subacute respiratory disease of chickens caused by *Haemophilus paragallinarum*. Clinical signs are generally mild, and the main impact of the disease is a reduction in egg production in laying flocks and poor performance in growing chickens (Blackall and Yamamoto 1998). Infectious coryza occurs worldwide, but the distribution of serovars varies from country to country (Sandoval; Terzolo, and Blackall 1994;Poernomo et al 2000;Bragg; Coetzee, and Verschoor 1996). Serovar B, as classified under the Page scheme, has not been isolated in Australia, but strains of serovars A and C are endemic (Blackall et al 1990) and controlled through the use of antibiotics and commercial vaccines. If serovar B were to enter Australia, new vaccination strategies would be required.

True vertical transmission of *H. paragallinarum* does not occur, but contamination of the shell by infected respiratory secretions is possible. Transmission of *H. paragallinarum* by this means is, however, considered very unlikely due to the fragility of the organism.

Detection methods

Diagnosis of the disease is based on clinical history and isolation of catalase negative, gram-negative organisms that exhibit a specific growth pattern (satellitic) and fail to grow in air. *H. paragallinarum* should be grown on blood or chocolate agar at 37 °C in 5% carbon dioxide, anaerobically or under reduced oxygen tension (Blackall and Yamamoto 1998). However, *H. paragallinarum* is a slow-growing, fastidious organism and is frequently overgrown by other faster growing commensal organisms (Blackall 1999). A PCR assay has been developed and can be applied to suspect colonies or directly to samples from the sinus of chickens as a molecular diagnostic test (Blackall and Yamamoto 1998).

Risk management

H. paragallinarum is not routinely tested under European Pharmacopoeia requirements. Although there is only a low likelihood of egg transmission and subsequent vaccine contamination, an appropriate level of confidence in freedom from contamination of the bulk/final vaccine is considered necessary to prevent the introduction of serovar B. It is therefore recommended that the bulk or final live avian vaccine be tested for the pathogen using an appropriate culture or other detection method.

Ornithobacterium rhinotracheale

O. rhinotracheale is a respiratory pathogen of avian species, and has been implicated in both primary and secondary infections in chickens and turkeys. In meat chickens infection results in mild respiratory signs, decreased growth rate and slight increases in mortality (van Veen; van Empel, and Fabri 2000). Infection with *O. rhinotracheale* may cause significant economic losses, especially in breeder birds. Infection with *O. rhinotracheale* has not been documented in Australia.

Egg transmission of *O. rhinotracheale* may occur, either trans-ovarially or by cloacal contamination. *O. rhinotracheale* has been isolated from the oviducts and ovaries of experimentally infected turkey breeder hens (Back et al 1998), and from egg shells and yolk sac of day-old birds, albeit with very low frequency (<1%) (van Empel and Hafez 1999).

Detection methods

The clinical signs and lesions are not pathognomonic. A commercial ELISA, IDEXX FlockChek *O. rhinotracheale* test kit, is available and will detect antibodies in both chicken and turkey serum.

Optimal growth of *O. rhinotracheale* occurs on 5% sheep blood agar in micro-aerophilic conditions (van Empel and Hafez 1999). A PCR test has been developed and is useful for identification of the organism and in epidemiological studies.

Risk management

O. rhinotracheale is not routinely tested under European Pharmacopoeia requirements. As egg transmission may occur, a subsequently contaminated avian vaccine could cause a multifocal outbreak with significant economic losses. It is

therefore recommended that the bulk or final live avian vaccine be tested for the pathogen using an appropriate culture or other detection method.

Mycoplasma spp

Mycoplasma iowae

Mycoplasma iowae is primarily a pathogen of turkeys, causing embryo mortality and reduced hatchability. However, infections of chickens can occur (Trampel and Goll 1994). There are many different strains of *M. iowae,* and marked within-species antigenic variation (Rhoades 1984). *M. iowae* occurs in North America, Europe, India and Asia, and is presumed to exist worldwide, with the exception of Australia and New Zealand.

M. iowae has been isolated from the oviduct of chickens and turkeys, and is known to be vertically transmitted in both species (Al-Ankari and Bradbury 1996).

Mycoplasma synoviae

Mycoplasma synoviae causes respiratory disease and infectious synovitis in chickens and turkeys (Marois; Dufour-Gesbert, and Kempf 2000). There is substantial variation in pathogenicity and tissue tropism among isolates of *M. synoviae*, with many strains causing subclinical disease, and some resulting in significant disease problems (Kang; Gazdzinski, and Kleven 2002;Stipkovits and Kempf 1996). Some strains of *M. synoviae* occur in Australia (Gilchrist and Cottew 1974;Morrow et al 1990).

Chickens, turkeys and guinea fowl are the natural hosts of *M. synoviae*, while ducks, geese, pigeons, Japanese quail, red-legged partridge, sparrows and pheasants have been found to be naturally infected without showing clinical signs of disease (Kleven 1997;Bradbury; Yavari, and Dare 2001;Yamada and Matsuo 1983;Kleven and Fletcher 1983).

M. synoviae is present in poultry-producing countries world-wide, including Australia. It is likely that variations in strain and pathogenicity occur between countries, but to date there has been no evidence presented that exotic strains of *M. synoviae* are more virulent than Australian strains.

Vertical transmission is an important means of spread of *M. synoviae*, with outbreaks in commercial flocks often being traced to infected breeder birds(Morrow et al 1990;Droual et al 1992;Ewing et al 1996). Commercial layers, especially those on multi-age complexes, are commonly infected with *M. synoviae* (Daft and Kinde 1990;Kleven 1999).

One study described the isolation of *M. synoviae* from a combination of embryonated eggs, dead-in-shell eggs and infertile eggs of chickens (Vardaman 1976). Egg transmission of Mycoplasma also occurs in naturally-infected ducks and geese (Bencina; Tadina, and Dorrer 1988a;Bencina; Tadina, and Dorrer 1988b). Transmission of *M. synoviae* has been demonstrated in both embryonated and infertile duck eggs (Bencina et al 1988a). Mycoplasmas can be transmitted via yolk-sac inoculation of embryonated eggs (Bradbury and Howell 1975).

Detection methods for Mycoplasma spp.

Tests for both *Mycoplasma gallisepticuma* and *M. synoviae* as per the European Pharmacopoeia for the SPF flocks (5% of flock tested monthly) are agglutination with positives confirmed with haemagglutination inhibition. A general *Mycoplasma* test is also applied to final bulk live avian vaccines.

Risk management for Mycoplasma spp.

Although there is a potential for transmission of *Mycoplasma spp* in eggs with subsequent contamination of vaccines, it is considered that current risk management measures provide adequate quarantine confidence. These measures include the routine surveillance and monitoring of SPF flocks for *M. synoviae*, in accordance with the European Pharmacopoeia and also testing of the bulk or final vaccine for *Mycoplasma spp* contamination in accordance with our current vaccine import policies.

RECOMMENDATIONS

1. That compliance with the European Pharmacopoeia requirements for SPF flocks be considered sufficient to address Australian animal quarantine concerns with:

    o   imported SPF eggs for *in vitro* laboratory use, and
    o   use in human vaccines[i], and
    o   inactivated veterinary vaccines

    subject to appropriate quarantine controls on importation, transport, storage, use and disposal.

2. That use of SPF eggs of non-Australian origin in live avian vaccines be permitted subject to additional assurances of safety as specified below, and subject to demonstration of a critical national need for the vaccine.

3. That the current contingency clause be removed from all end uses other than production of live avian vaccines[ii].

    Contingency clauses may not be fully compliant with Australia's international trading obligations. However, the justification for the contingency clause for use in live avian vaccines is that:
    • use in vaccines represents an extremely high risk and there is an international history of problems caused by use of contaminated SPF eggs especially in live avian vaccines
    • there is currently only one SPF egg producer in Australia and any significant shortfall in production and supply may make importation necessary for essential services
    • If this producer is unable to meet demand, it may be impractical for users of small numbers of SPF eggs to import batches from overseas

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[i]   This review only considers animal quarantine issues. Human health issues are the responsibility of Commonwealth and State/Territory departments of health and the TGA.

[ii]   Reference to live avian vaccines includes other avian therapeutics which have not undergone a pathogen inactivation step.

(this includes eggs for human vaccine production, biomedical research, disease surveillance, etc)

- However, availability of SPF eggs is critical to national human and animal health and well being.

4. That a "sunset" clause be applied to the policy removing the contingency clause from the conditions for use of SPF eggs of non-Australian origin in live avian vaccine production. This would allow issues such as the additional testing of final live avian vaccines to be resolved. A period of 12 months is proposed.

5. For those pathogens identified by this review as requiring additional sampling and testing of the SPF flock or more sensitive detection tests on the final live avian vaccine, above and beyond international standards such as the European Pharmacopoeia:

   a) That additional testing (ie increased sample size) of the SPF flock is required within the 21 day pre-egg collection period for the following diseases to provide at least a 99% confidence of detecting disease at a 0.5% prevalence level (after taking sensitivity of the diagnostic test into account)
      Avian influenza virus
      Newcastle disease virus
      Avian paramyxovirus-2
      Avian paramyxovirus-3
      Avian pneumovirus (Turkey viral rhinotracheitis)
      Avian adenovirus group 1
      Infectious bronchitis virus
      Avian reovirus.

   b) That the bulk or final live avian vaccine be tested for both *Ornithobacterium rhinotracheale* and *Haemophilus paragallinarum* using an appropriate culture or other detection method.

6. That a further review be undertaken on sensitive extraneous agent detection methods. This will require:

   - technical input from the various vaccine manufacturers, poultry industry bodies, Australian Veterinary Poultry Association, Australian Animal Health Laboratory, AusBioTech and other organizations with an interest in the issue of SPF egg importation.

7. Once the review on more sensitive extraneous agent testing is completed, testing on live avian vaccines, determined by the review to be appropriate, may be available to vaccine manufacturers as an alternative to the additional sampling and testing of the source flock as described in point 5a. above.

8. Until the review on more sensitive extraneous agent testing is completed, the option of additional highly sensitive extraneous testing on live avian vaccines as an alternative to additional sampling and testing of the source flock would only be available to vaccine manufacturers on a case by case basis. Approval will require

detailed justification including test sensitivity at very low titre levels. Such testing will also be specifically identified as an issue in the public consultation currently required on individual imported livestock (including avian) vaccines.

9. Vaccine manufacturers and the APVMA are encouraged to implement additional controls on endemic pathogens (eg chicken anaemia virus and avian leucosis virus) in relation to Australian SPF flocks and/or live avian vaccines produced in Australia.

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Molecular detection methods for exotic avian viral pathogens
Summary of references

Avian Influenza Virus

Although virus isolation using embryonated eggs is considered to be the gold standard, RT-PCR-ELISA has been demonstrated to be a very effective technique to detect avian influenza virus, including low-pathogenicity strains (Dybkaer et al 2003). The PCR-ELISA is about 100 times more sensitive than detection of PCR products using agarose gel electrophoresis and comparable to virus propagation in eggs (Munch et al 2001). A multiplex PCR has also been developed to detect avian influenza virus along with 5 other avian respiratory pathogens (Pang et al 2002). A real time reverse transcriptase PCR has also been developed which is directed to regions of the AIV matrix gene that are conserved among most type A influenza viruses (Spackman et al 2003).

Avian Adenoviruses

Diagnostic services for a generic FAdV serotypes 1-12 using PCR are available. A PCR capable of amplifying avian adenovirus 421-bp DNA product from the 12 serotypes of Group I and serotypes from Group II and III has been developed (Xie et al 1999). A PCR has also been developed specifically for serotype 4 (Ganesh; Suryanarayana, and Raghavan 2002).

Avian Pneumovirus (Turkey Rhinotracheitis virus)

A nucleocapsid based RT-PCR detected 21 of 21 strains of TRTV (Bayon-Auboyer et al 1999). A RT-PCR utilising primers developed from the matrix (M) gene sequence of the Colorado strain of virus will detect US isolates but not other pneumoviruses (Pedersen et al 2001;Shin et al 2000).

Infectious Bronchitis Virus

A sensitive and specific nested RT-PCR has been developed to detect IBV in tissue utilising a well conserved region of the nucleocapsid gene (Falcone et al 1997). General oligonucleotide primers based on highly conserved sequences from the spike protein gene (S-1) have also been used to detect IBV by RT-PCR regardless of serotype (Keeler et al 1998). A multiplex PCR has also been developed to detect infectious bronchitis virus along with 5 other avian respiratory pathogens (Pang et al 2002).

Avian Reovirus

A reverse transcriptase PCR technique, which amplifies a fragment of the reovirus L1 gene segment, has been demonstrated to be capable of detecting 44 of 44 mammalian reovirus field isolates (Leary et al 2002). However, the test would need to be validated for avian strains. A RT-PCR amplifying a 672-base pair fragment of the S3 segment of avian reovirus was capable of detecting 9 strains indicating that this segment is well conserved for avian reovirus (Lee; Shien, and Shieh 1998). A broad RT-PCR amplifying a 538 base pair fragment of the Sigma 2 gene has been developed to detect reoviruses in environmental seawater samples. It is interesting to note that this RT-PCR test detected reovirus in 14 out of 72 samples compared with 8 using a polyacrylamide gel electrophoresis and none using a haemagglutination test or relying on cytopathic effects in supposedly sensitive cells (Muscillo et al 2001). A reovirus specific 532 base pair amplified using primers from the S1 gene of avian reovirus has been used to detect 6 reference strains and 23 field isolates of avian reovirus (Xie et al 1997).

Newcastle Disease Virus

RT-PCR has been applied to detection of NDV in poultry vaccines using 2 primer pairs spanning the cleavage site of the F0 fusion protein coding sequence. Sensitivity was 5×10^2 EID_{50} in live vaccine (Bruckner et al 1996). A multiplex RT-PCR assay for NDV and avian pneumovirus has also been described (Ali and Reynolds 2000). A RT-nested PCR designed from the consensus fusion gene sequence and coupled with an ELISA has been demonstrated to be a sensitive and specific method to detect the presence of all pathotypes of NDV (Kho et al 2000). A multiplex PCR has also been developed to detect NDV along with 5 other avian respiratory pathogens (Pang et al 2002).

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