British Poultry Science Volume 51, Number 1 (February 2010), pp. 1–11

Taylor & Francis
Taylor & Francis Group

GORDON MEMORIAL LECTURE

Viral respiratory diseases (ILT, aMPV infections, IB): are they ever under control?[1]

RICHARD C. JONES

School of Veterinary Science, University of Liverpool, Neston, South Wirral, England

Abstract 1. The use of vaccines is the main approach to control of the economically important poultry viral respiratory diseases infectious laryngotracheitis (ILT), avian metapneumovirus (aMPV) infections and infectious bronchitis (IB). This paper appraises the current methods of vaccine control in the light of the nature of each virus and epidemiological factors associated with each disease.

2. Infectious laryngotracheitis virus (ILTV) exists as a single type with a wide range of disease severity. It is a serious disease in certain regions of the world. Recent work has distinguished molecular differences between vaccine and field strains and vaccine virus can be a cause of disease. Vaccines have remained unaltered for many years but new ones are being developed to counter vaccine side effects and reversion and reactivation of latent virus.

3. Avian metapneumoviruses, the cause of turkey rhinotracheitis and respiratory disease in chickens exists as 4 subtypes, A, B, C and D. A and B are widespread and vaccines work well provided that accurate doses are given. Newer vaccine developments are designed to eliminate reversion and possibly counter the appearance of newer field strains which may break through established vaccine coverage.

4. IB presents the biggest problem of the three. Being an unstable RNA virus, part of the viral genome that codes for the S1 spike gene can undergo mutation and recombination so that important antigenic variants can appear irregularly which may evade existing vaccine protection. While conventional vaccines work well against homologous types, new strategies are needed to counter this instability. Molecular approaches involving tailoring viruses to suit field challenges are in progress. However, the simple use of two genetically different vaccines to protect against a wide range of heterologous types is now a widespread practice that is very effective.

Correspondence to: Professor R.C. Jones, School of Veterinary Science, University of Liverpool, Leahurst Campus, Neston, South Wirral CH64 7TE, UK. E-mail: jonesrc@liv.ac.uk

[1]This lecture, in memory of Dr R.F. Gordon, is the 26th in the series and was delivered at the Spring Meeting of the UK Branch of the World's Poultry Science Association at Southport, Merseyside on 31st March 2009.

ISSN 0007-1668(print)/ISSN 1466-1799 (online)/10/010001-11 © 2010 British Poultry Science Ltd
DOI: 10.1080/00071660903541378

5. None of the three diseases described can claim to be satisfactorily controlled and it remains to be seen whether the newer generations of vaccines will be more efficacious and cost effective. The importance of constant surveillance is emphasised and the testing of novel vaccines cannot be achieved without the use of vaccine-challenge experiments in poultry.

INTRODUCTION

Viral respiratory diseases of poultry constitute an important cause of economic loss to the poultry industry worldwide in terms of impaired growth, reduced egg production and quality, mortality, slaughter downgrading and several ancillary factors including diagnoses, vaccines, and antimicrobials to treat inter-current bacterial infections etc. Those diseases with the potential to cause the most severe losses in terms of mortality are very virulent Newcastle disease and highly pathogenic avian influenza. Three other viral respiratory diseases constitute what might be called the ''second tier'' of importance in terms of disease severity, namely infectious laryngotracheitis (ILT), avian metapneumovirus (aMPV) infections (the cause of infections turkey rhinotracheitis, avian rhinotracheitis) and infectious bronchitis (IB). Of these, IB is the most economically important and in the UK alone, it has been reported to be responsible for losses amounting to some £23 million annually. For all three, disease severity can be exacerbated and prolonged by concurrent infections with bacteria including *E. coli* and *Mycoplasma gallisepticum* or if the birds are immunosuppressed by infectious bursal disease or chicken anaemia virus infection. However, this paper will concentrate on the uncomplicated infections alone.

For all three, live vaccines are available and widely used but control is variable. This paper attempts to assess how successful control by vaccination is, taking into account the nature of the viruses and the different epidemiological features which are influential in this respect.

The viruses

The three viruses are unrelated, belonging to different groups in the virus classification. Infectious laryngotracheitis virus (ILTV) is an alpha-herpes virus assigned to the *Iltovirus* genus (Figure 1). ILT was one of the earliest diseases described for poultry (1925) and affects chickens and pheasants but rarely turkeys. Avian metapneumovirus (aMPV, Figure 2) belongs to the *Paramyxoviridae* family and is the cause of turkey rhinotracheitis, originally reported in South Africa in the late 1970s, but it affects chickens also and is the ''youngest'' of the three. IB, caused by a type 3 coronavirus (Figure 3), was first reported in 1931 but still flourishes. Despite years of attempting to control the diseases caused by these viruses by the use of vaccines, they are still present.

INFECTIOUS LARYNGOTRACHEITIS

ILT occurs in chickens with a wide spectrum of disease severity, ranging from subclinical to

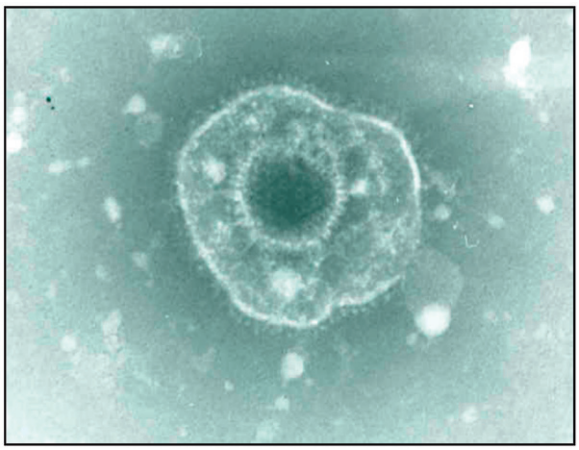

Figure 1. *Electron micrograph of the herpesvirus of infectious laryngotracheitis. The icosahedral virion measuring 100 nm diameter is surrounded by a lipoprotein envelope. [Negative staining].*

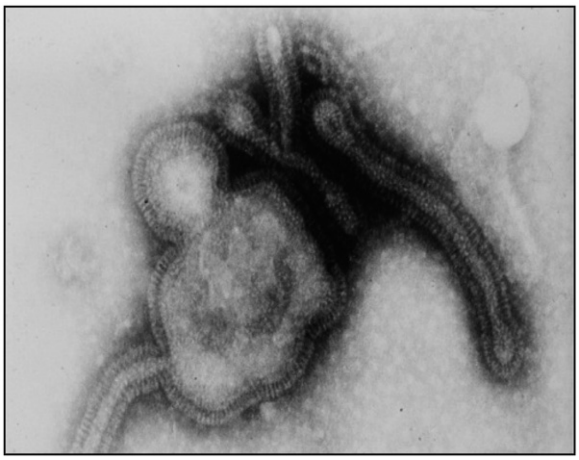

Figure 2. *The pleomorphic appearance of avian metapneumovirus particles under the electron microscope. The surface is covered by a fringe of even-width spikes (peplomers). The large particle measures approximately 100 nm across. [Negative staining]*

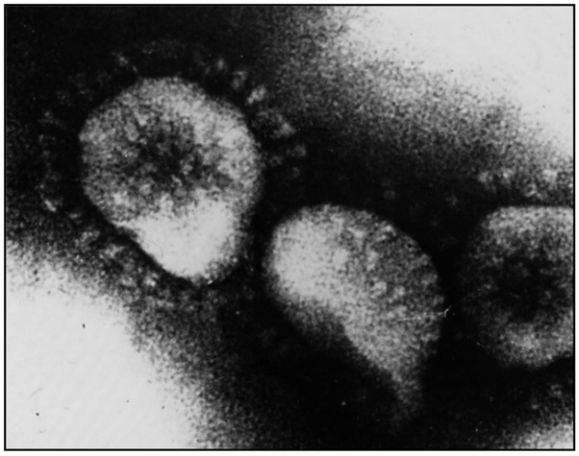

Figure 3. *Infectious bronchitis virus particles, each measuring 100–140 nm diameter. The surface of each particle is covered in club-shaped spikes, which are important in cell attachment, virus neutralisation and genotyping. [Negative staining].*

Table. *Recent molecular groupings of ILTV strains in Europe, USA, Australia and percentage of outbreaks determined to be caused by vaccine viruses*

Region	Molecular groupings	% of outbreaks vaccine-related
Europe – 35 years (Neff *et al.*, 2008)	3 ''clones''	94
USA Garcia *et al.*, 2007–2009)	9 ''genotypes''	63
Australia (Kirkpatrick *et al.*, 2006)	5 ''genetic groups''	Most unrelated to vaccines

peracute with significant mortalities (Guy & Garcia, 2008). Unlike IB and aMPV infections, ILT appears to solely affect the respiratory tract and does not spread rapidly. Disease, sometimes very severe, occurs in certain regions of the world where it usually remains endemic, while in other countries it is unknown or of little importance.

Epidemiological features

ILTV exists as a single serotype, an advantage from the point of view of control by vaccines and this is also helpful in serological diagnosis. Different strains (e.g. wild and vaccine viruses) can be differentiated by molecular methods.

One aspect of ILT epidemiology relating to its being a herpes virus is that it becomes latent within the chicken (Guy & Garcia, 2008). Essentially this means that when affected birds have recovered from the acute phase of infection, the virus assumes a non-infectious state and resides in the trigeminal ganglia (Williams *et al.*, 1993), though the flock appears to be in normal health. Live ILT vaccines can also become latent (Hughes *et al.*, 1991). The consequences are that latent virus can become reactivated by factors such as stress or onset of lay (Hughes *et al.*, 1989), so that such a flock can become an unrecognised source of infection (Williams *et al.*, 1992). There are no known wild bird reservoirs of this virus and there is no evidence that it is egg transmitted (Bagust & Johnson, 1995).

Control is by the use of live vaccines in endemic areas and flock owners are ''afraid to stop'' vaccinating. Initial studies hinted that promoted by the nature of latency, ILT vaccines themselves were responsible for some outbreaks of disease in naive flocks. With improved molecular techniques, it has now been confirmed that this does happen, but other field strains of virus are unrelated to vaccine strains (Ojkic *et al.*, 2006; Oldoni *et al.*, 2008; Neff *et al.*, 2008).

Recent findings

Recent molecular investigations have shown differences between strains of ILT viruses in different regions. It is worth pointing out that the molecular groupings have been given different labels according to the genetic comparisons (Table). In Europe, a study of strains collected over 35 years showed that 94% (98/104) of strains were related to vaccines (Neff *et al.*, 2008). A similar study in the USA (Oldoni *et al.*, 2009) found that of 9 ''genotypes'' reported, 63% were related to vaccine strains. In Australia, 5 ''genetic groups'' have been established but in contrast, most ILT was considered not to be due to vaccine types (Kirkpatrick *et al.*, 2006). New strains have been reported to appear recently in Australia and ILT is considered an ''emerging disease'', but no one knows from where they have originated.

Control of ILT

Empirical attenuated ILT vaccines either of chick embryo origin (CEO) or tissue culture origin (TCO) have been used for many years with success (Guy & Garcia, 2008). Some time ago we discovered that the commercial vaccine used in the UK behaved in the same way as a field virus, that is, it had the same latency characteristics as wild type ILTV in the bird and persisted for life (Hughes *et al.*, 1991). Thus, it was perceived that a significant proportion of disease could be due to vaccine virus becoming ''wild'' and recently referred to in the USA as ''vaccinal laryngotracheitis''. Molecular analysis, as shown above, has confirmed that this is the case. However, Korean workers found that one of four conventional ILT vaccines protects against virus latency (Han & Kim, 2003). Despite the presence of ILTV strains genetically different from vaccine strains,

Rodríguez-Avila *et al*. (2008) in the USA have recently shown that conventional vaccines are effective in protecting against them. Thus it appears that the fine genetic differences which differentiate field from vaccine viruses and which are helpful in epidemiological studies are not sufficiently different to require radically new vaccines

Problems associated with conventional ILT vaccines have included vaccinal reactions, especially where other respiratory agents are present, reversion of vaccine viruses to virulence and latency with later reactivation. In addition, because ILT vaccines are not usually given until 10 weeks of age or later, birds are vulnerable up to this point. As a result, several new approaches have been explored to produce a new generation of safer vaccines. These include the production of a glycoprotein G-deficient vaccine (Devlin *et al*., 2008), G being associated with latency and a fowl pox vector carrying ILTV genes (Davison *et al*., 2006) or glycoprotein G together with the haemagglutinin of Newcastle Disease virus (Sun *et al*., 2008). It remains to be seen whether vaccines of this type will prove to be more efficacious than the empirically derived vaccines.

A new vaccine recently trialled in USA and South America consists of the Marek's disease turkey herpesvirus (THV) vaccine used as a vector containing ILTV genes gD and gI (I. Tarpey, personal communication). The vaccine is given by injection at one-day-old and since the THV vaccine persists in the chicken, it is claimed to give lifelong immunity against ILT. Because it contains only defined ILT genes, there are no problems with respiratory reactions or latency.

Finally, it is worth noting that Bagust and Johnson (1995) suggested that ILTV could be a suitable candidate for eradication, since it has a number of properties appropriate for this approach to control. These include its existence as a single serotype, lack of vertical transmission and no apparent wildlife reservoir. They proposed the use of a genetically defined marker vaccine such as mentioned above, together with a matched ELISA. The latter would distinguish vaccinal response from field infection and permit differentiation of birds which are naturally infected from those given vaccine.

Conclusions on ILT control

ILTV viruses have stayed remarkably stable over the years compared to say IBV, but they can now be distinguished by molecular methods. ILT continues to cause disease problems, especially in the Americas and Australia. Over the years, vaccines, derived from egg or tissue culture passage, have remained mostly unaltered but there have been problems with vaccine reaction, reversion of vaccine virus to virulence and latency.

New genotypes different from vaccines have emerged, particularly in Australia. In the USA, conventional vaccines have been shown to be effective against genetic variants (Rodríguez-Avila *et al*., 2004) but will future variants emerge which evade vaccinal protection? Another question is, since no known wildlife reservoirs are recognised, from where do the variants originate? To date, ILT may be considered to have been restrained rather than well controlled. Perhaps the new vaccines such as the turkey herpes vector vaccine will allow much better control.

AVIAN METAPNEUMOVIRUS INFECTIONS

Avian metapneumovirus is the cause of turkey rhinotracheitis (TRT), first seen in South Africa in the 1970s (Buys *et al*., 1989). It was soon shown to cause respiratory disease in chickens too (avian rhinotracheitis, ART) and sometimes swollen head syndrome (SHS) (Gough & Jones, 2008). aMPV soon spread to Europe and the Middle East and most parts of the world where commercial poultry are kept, apart from Australia and North America. Initially, two subtypes of virus were recognised, A and B, based on differences in the surface glycoprotein gene sequences (Juhasz & Easton, 1994). In 1996, a third subtype, C was identified in the USA in turkeys in Colorado and later in the important turkey producing state of Minnesota (Seal, 1998). In the USA, the disease is called ''avian pneumovirus infection of turkeys'' and subtype C does not appear to be important in chickens. Subsequently, a subtype D was described in France (Bäyon-Auboyer *et al*., 2000) and a variant C appeared in ducks in the same country (Toquin e*t al*., 2008). Surprisingly, a subtype C has been reported in South Korea in pheasants in a market (Lee *et al*., 2007). aMPV primarily affects the respiratory tract, especially the upper tissues (Figure 4), and is able to replicate in the oviduct, the latter resulting in loss of egg production and quality (Gough & Jones, 2008).

Epidemiological features

Since the first reports of aMPV in South Africa and its subsequent appearance in most parts of the world, the mode of spread of these viruses has been a source of conjecture. How the virus reached Europe, the Middle East and further afield from South Africa is unknown. One suggestion has been transmission by long-distance migratory birds (Jones, 1996), although efforts to find evidence of infection in wild

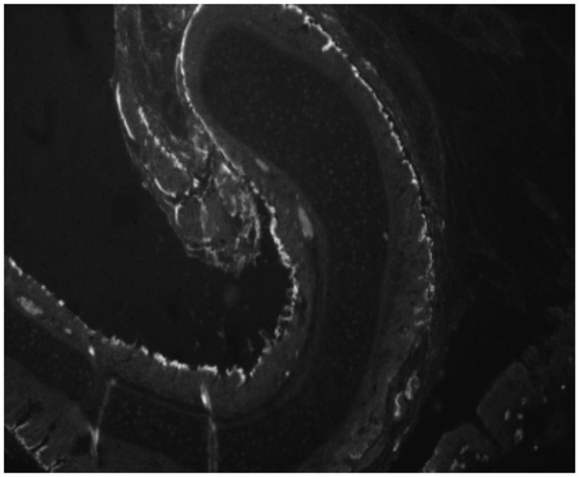

Figure 4. *In uncomplicated infections, avian pneumoviruses principally affect the upper respiratory tracts in the chicken and the turkey. Immunofluorescence staining shows the presence of virus (white) in the epithelial cells of a section of turkey turbinate.*

European species have not been fruitful. Perhaps even more puzzling is the fact that while subtype C has been present in turkeys in the USA, principally Minnesota, with its many lakes, while A and B are present in Central and South America, there is no apparent mixing of subtypes in the two regions, despite the migratory flight paths of wild waterfowl between the north and south. Evidence of aMPV infection in wild birds has been reported in the USA (Shin *et al*., 2002; Turpin *et al*., 2008), but this in itself does not mean that infected species are able to transmit infection to susceptible poultry.

The isolation of aMPV from very young turkeys (Gough *et al*., 1988) and the presence of abundant amounts of virus in the oviducts of infected laying turkeys (Jones *et al*., 1988) suggest that egg transmission may occur from time to time.

Control of aMPV infections

The main approach to control of aMPV infections is by the use of live attenuated and killed vaccines, the former derived by attenuation in cell cultures or fertile eggs and tracheal organ cultures (Cook & Ellis, 1990; Williams *et al*., 1991). In chickens, live vaccines are incorporated into the vaccination programme at a different time from IB vaccines, since interference occurs (Cook *et al*., 2001), although aMPV vaccination simultaneously with Newcastle disease vaccines does not adversely affect the efficacy of either (Ganapathy *et al*., 2005). Killed vaccines are given by injection before the onset of lay but only after priming with live vaccines. Except for the USA, where subtype C is present, A and B are the prevalent subtypes elsewhere and vaccines against each of these two give good protection against the other (Cook *et al*., 1995). A and B type vaccines also give some protection against subtype C but not vice versa (Cook *et al*., 1999).

A recent survey of aMPVs in chickens and turkey flocks in Western Europe showed that subtype B predominates in the 6 countries studied, perhaps reflecting the wider use of subtype B vaccines (R.C. Jones and K.J. Worthington unpublished). This survey did not distinguish between vaccine and field viruses. Subtype A and B vaccines have been used successfully in most countries and in both species, where the disease is prevalent. In spite of this, several reports suggest that there is room for improved vaccines or at least closer attention to accurate vaccine delivery. For example in turkeys, some weeks after aMPV vaccination, respiratory disease sometimes occurs which has been diagnosed as TRT. This phenomenon been confirmed to be due to vaccine virus becoming virulent after recycling among birds which did not receive a dose of vaccine originally (Catelli *et al*., 2006).

A recent Israeli longitudinal study of aMPV infection in a small number of chicken and turkey flocks revealed that both vaccine and field strains of aMPV A and B subtypes could be recovered simultaneously (Banet-Noach *et al*., 2009). Recently in Brazil (Villarreal *et al*., 2009) and Italy (Cecchinato *et al*., 2009), field evidence has suggested that the existing vaccines are not fully effective against new strains of virus.

Though the empirical vaccines have been effective where given accurately, moves have been made to formulate novel vaccines which do not revert to virulence. These include vector vaccines, subunit vaccines and DNA vaccination – all only partially effective. One approach has involved the use of reverse genetics to make an infectious clone and looks promising (Naylor *et al*., 2004; Ling *et al*., 2008; C.J. Naylor and P. Brown, personal communication). *In ovo* vaccination has been shown to be a practical and effective form of delivery for protecting turkeys and chickens against aMPV infection (Worthington *et al*., 2003; Tarpey & Huggins, 2007).

Finally, in regions where flock density is low, it is apparent that aMPV infections can be eradicated with careful monitoring and strict attention to biosecurity. Examples are Sweden and Colorado, where subtype C was first reported in the USA. However, in the state of Minnesota, where C gained a strong foothold in the immense turkey industry, the disease has all but been eradicated (K. Nagaraja, personal communication). Serological monitoring has shown no evidence of aMPV for some time and the turkey industry has stopped vaccinating.

No clinical cases and no isolations have been made for more than 4 years.

Conclusions on aMPV disease control

A small number of subtypes exist, A, B, C and D. D is rare, C appears to be important only in the USA, perhaps less and less so. A and B are the most widespread and important ones. Current vaccines are successful if administered accurately to each bird but problems can arise with reversion and there is evidence of possible virus evolution. New safer vaccines are being developed. However, the work of Banet-Noach *et al.* (2009) in Israel indicates that wild type virus can still be present in vaccinated flocks, illustrating that control is less than satisfactory with present vaccines.

INFECTIOUS BRONCHITIS

Infectious bronchitis is the most important of the viral respiratory diseases to be discussed here. It is considered to be the most important endemic viral disease of poultry in countries where highly pathogenic avian influenza or virulent Newcastle disease are not present (Cavanagh & Gelb, 2008). It is a disease of chickens and not turkeys. In addition to being a respiratory disease, affecting all ages and affecting growth, it is one of the most important causes of loss of egg production and quality (Cavanagh & Gelb, 2008). Some strains have a predilection for the kidneys and cause significant mortalities in young birds (Dhinakar Raj & Jones, 1997*a*). There is some evidence that IBV may sometimes be associated with enteritis and infertility in males (Villarreal *et al.*, 2007*a*, *b*). Control of IB is by the use of live attenuated and killed vaccines. However, IB presents unique challenges regarding its control. Genomic diversity and the ability of the virus to rapidly change have created different genotypes of the virus that evade protection by conventional vaccines (Cavanagh & Gelb, 2008). Approaches to counteract this problem are discussed below.

The virus

The cause of IB is a type 3 coronavirus. The virus, infectious bronchitis virus (IBV) is an enveloped RNA virus with a surface fringe of club-shaped spikes. The spike is in two parts, S1 and S2 and the gene coding for the S1 spike has a hypervariable region which is liable to mutate or undergo reassortment with other IBVs, thus generating novel variant IBVs. The S1 spike is important in viral attachment, is a major component in development of immunity and has traditionally been important for cross-neutralisation, the original basis for IBV classification. The different types of IBV generally give little or no protection against heterologous types, although there are some exceptions. Important variants appear from time to time in a very unpredictable manner and are a constant challenge to vaccine strategies. Hence there is a constant need for surveillance of prevalent types.

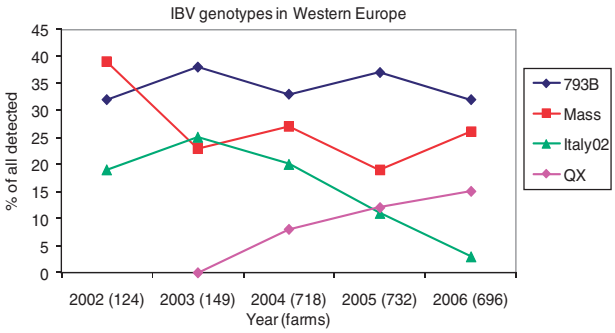

Figure 5. *Prevalence of the main infectious bronchitis genotypes in Western Europe (UK, Belgium, France, Holland, Germany and Spain) between 2002 and 2006 detected by RT-PCR and sequencing of the S1 spike gene. 793B variant (including 4/91 and IB88 vaccines) and Massachusetts types predominate. Of the new genotypes, Italy 02 appears to be in decline, while QX is in the ascendancy. Different countries show distinct differences in prevalence of types (from Worthington* et al.*, 2008).*

In a recent survey of IBV in some 5000 commercial chicken flocks in UK, France, Spain, Holland, Germany and Belgium between 2002 and 2006 (Worthington *et al.*, 2008), 59% were positive for IBV. The most prevalent genotypes were Massachusetts and the major variant 793B (4/91 or CR88) (Figure 5). On the basis of nucleotide sequence shared identity, approximately 50% of each were considered to be vaccine virus (H120, MA5, 4/91, IB88, etc). The next most common genotypes were two new important field types: Italy 02 and a virus originally detected in China called QX (Worthington *et al.*, 2008). The origins of Italy 02 are unknown (Dolz *et al.*, 2006) but QX originated in China in 1996 (Wang *et al.*, 1998), though how it arrived in Europe is unknown. Italy 02 has behaved primarily as a respiratory pathogen, but QX has had more serious consequences, causing nephritis in young flocks and false layers in egg laying flocks. This survey is an example of the diversity of IBV genotypes in a region and the appearance of new ones. Considerable diversity has been well documented for USA, Australia and China and in recent years, reports of novel genotypes have been published from Brazil (Brandao *et al.*, 2009), Argentina (Rimondi *et al.*, 2009), Thailand (Pohuang *et al.*, 2009), Malaysia (Zulperi *et al.*, 2009) and Africa (Ducatez *et al.*, 2009).

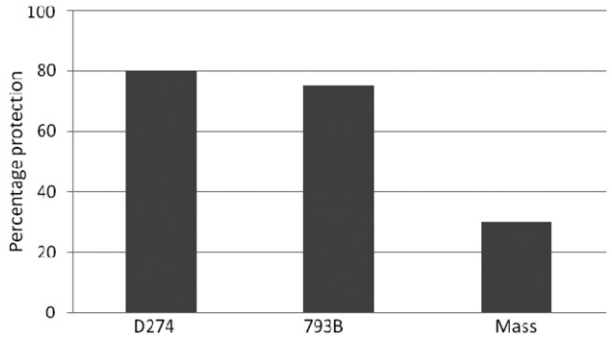

Figure 6. *An example of good protection offered by one genotype of live infectious bronchitis vaccine (D274) against an unrelated genotype (793B). Chicken were vaccinated at one-day-old with D274 vaccine and challenged at 21 d of age with D274, 793B or Massachusetts types. Five days later chicks were killed and protection was assessed according to the ciliary activity in tracheal sections. Protection against another different strain (Mass) was poor (from Dhinakar Raj & Jones, 1996).*

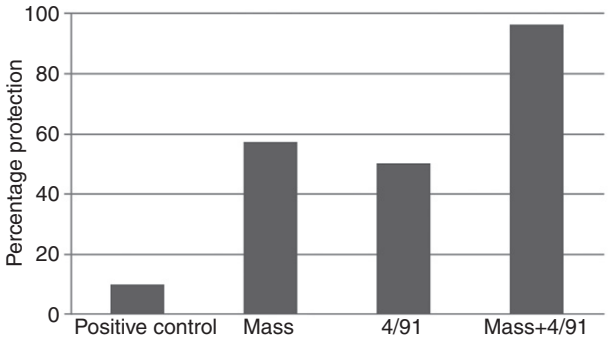

Figure 7. *The use of two heterologous vaccines (Massachusetts H120) and 4/91 gives good protection against a strain of IBV unrelated to either (here A1121). Protection against this virus by either vaccine alone is poor. Protection was assessed as in Figure 6 (from Cook* et al.*, 1999).*

Epidemiological features

IB viruses affect chickens but not turkeys like aMPV although turkeys and pheasants have their own different coronaviruses. IBV is not generally considered to be egg transmitted and while there is abundant virus in the oviduct of the hen shortly after infection (Jones & Jordan, 1972), very few chicks would be likely to hatch with virus.

Undoubtedly the biggest problem of control relates to the diversity of types and the appearance of new ones from time to time. Although in Europe, the number of important variants to appear in the last 50 years has been relatively small – Dutch types in the 1970s, 793B in the 1990s and two in this decade, Italy 02 and QX – their emergence is unpredictable. Perhaps increasing poultry populations and greater use of vaccines will promote the more rapid generation of new types.

Some well-established genotypes are found worldwide (e.g. Massachusetts) and in part due to the widespread use of vaccines. However, we are largely ignorant as to how IBVs move around the world. For example, QX originated in China but is now established in Europe. It travelled across Asia in a similar time span to that taken by avian influenza H5N1 but, as far as we know, without the mediation of wild birds. There is increasing evidence that wild birds can be infected with coronaviruses (Jonassen *et al.*, 2005; Woo *et al.*, 2008) but with few exceptions (Liu *et al.*, 2005; Sun *et al.*, 2007; Hughes *et al.*, 2009) they seem to be unrelated to IBV. As yet, there is no hard evidence that IBV is transmitted over long distances by wild species. Long-term persistence of IBV has been demonstrated after early infection but its significance is unknown (Jones & Ambali, 1987; Dhinakar Raj & Jones, 1997*b*).

Control of IB – how to deal with variants?

In regions where licensed vaccines are from the same genotype as the field challenge, then IB vaccines work well. However, where variants appear and persist and against which existing vaccines do not protect, then this presents a big problem. A number of possibilities exist for addressing the problem of a new important variant for which a new vaccine is perceived to be necessary.

(1) Test the existing repertoire of vaccines. Sometimes protection can be offered by an unrelated vaccine. For example, D274 vaccine protects against 793B variant (Figure 6).
(2) Develop an empirical vaccine by attenuation in eggs or perhaps cell culture. This is the traditional ''fire brigade'' method but is a long tedious process.
(3) Use molecular techniques to engineer a vaccine for the new challenge virus that will not revert to virulence. Recent examples of this are modified DNA vaccination (Tian *et al.*, 2008; Tan *et al.*, 2009) and a multi-epitope-based peptide vaccine (Yang *et al.*, 2009). One of the most promising of the new generation of vaccine appears to be the ''spike swapping'' technology, whereby using reverse genetics, an infectious clone is produced into which specific S1 spike genes can be incorporated, appropriate to the new variant (Casais *et al.*, 2001). Variations on this theme have also included incorporation of nucleocapsid genes or specific cytokines to induce a broader immunity (Cavanagh *et al.*, 2007).
(4) Use two heterologous IBV vaccines (Cook *et al.*, 1999). This method has been shown to be very successful, even though the mechanisms have not been elucidated to date. It is usual to first administer a Massachusetts-type vaccine followed by a variant (793B-type in Europe) offers wide protection against types which are different again (Figure 7). Such a programme

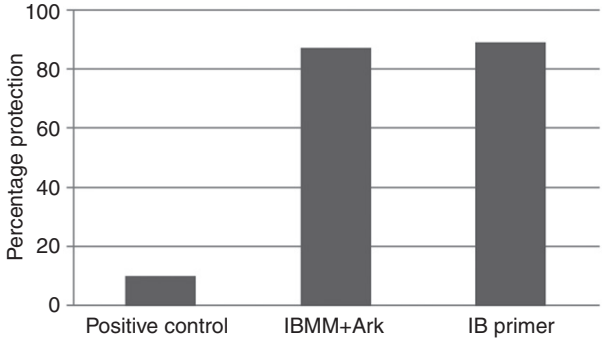

Figure 8. *A further example of the use of combinations of two live IBV vaccines at day old giving good protection, this time against the recent European variant Italy 02. IBMM+Ark comprises a Massachusetts vaccine given simultaneously with Arkansas vaccine, while the IB primer comprises Massachusetts vaccine in combination with D274. The protocol was again assessed as in Figure 6 (from Jones* et al*., 2005).*

has been shown to be efficacious against for example Italy 02 and QX (Jones *et al*., 2005, Figure 8); (Terregino *et al*., 2008; De Wit & van de Sande, 2009).

(5) *In ovo* vaccination. This is under development (Tarpey *et al*., 2006) and is dependent on the strain of IBV not killing embryos.

Conclusions on IB control

IB is highly infectious and maintaining virus-free flocks seems impracticable. The main choice for future strategies in IBV control seems to be as follows: (i) production of a specific tailored vaccine, produced by molecular technology and designed to protect against the variant challenge virus. This has the advantage that once the virus ''carrier'' is established, mechanisms for the insertion of the appropriate S1 spike gene (perhaps with accessory genes for cytokines etc., are now available. However, the question arises as to how many tailored vaccines the bird can take, if the numbers of variants in the challenge become large. (ii) The use of two different vaccines. This is simple to employ but is not guaranteed to succeed in every case and the mechanisms have not been fully established. However, unless a ''pan-IBV'' vaccine was to be produced (which is unlikely), this is a significant and very successful ''stop-gap'' approach. IBV shows viral evolution in action and is likely to remain one step ahead.

CONCLUDING REMARKS

Three viral respiratory diseases have been considered and they present different problems of control by vaccines. ILTV affects the chicken not the turkey, exists as a single serotype, but strains show differences in virulence. Genetic differences can be demonstrated but it is not clear how important they are in vaccine formulation. Vaccines have remained relatively unchanged but improved ones are needed for avoidance of vaccine reactions and problems of long immunity. In some countries, new strains are emerging but their origin is unknown.

Four subtypes of aMPV exist. A and B are virtually worldwide, affecting chickens and turkeys. While existing vaccines are effective when given accurately, field evidence of reversion and perhaps virus evolution highlights the need for improved safer vaccines.

IB presents the biggest challenge, with erratic and unpredictable appearance by variants which may evade protection of existing vaccines. Likely solutions to vaccine control may be tackled by specialised type-specific engineered vaccines or by the use of double heterologous vaccination. But what is the answer in the long-term? IBV variants will continue to arise: perhaps this disease will never be completely controlled.

Because of the difficulties outlined above, it cannot be claimed that any of the three diseases is satisfactorily controlled by vaccination. The ideal scenario where producing a vaccine against a new disease means the end of the problem does not apply in reality, even though experimental trials sometimes produce perfect results, with sterile vaccination as the outcome. The field situation is quite different.

For all three viruses, despite the widespread use of live vaccines, there is evidence, mediated through refined molecular techniques, of wild virus in circulation. Poor vaccine delivery, overwhelming field challenge, intercurrent infections, viral evolution, new variants from outside the immediate regions all conspire to militate against vaccinal efficacy. Existing vaccines may be described as restraining rather than fully controlling these diseases. It remains to be seen whether the new generation of vaccines will be more satisfactory than the existing empirical ones and cost effective. I think we can expect a move to *in ovo* delivery because of its convenience, even though it has not yet been taken up widely in Europe.

Some final thoughts. Essential to good control is constant surveillance using the optimal techniques available for detecting viruses currently infecting poultry populations, with the added benefit of alerting diagnosticians to possible new variants of current viruses or indeed new disease agents. It must also be remembered that vaccine efficacy can only be determined by the use of vaccine-challenge experiments in birds.

ACKNOWLEDGEMENTS

I should like to thank all my colleagues and collaborators including postgraduates and technicians who have worked with me on these viruses over the years. I am especially indebted to Professor Frank Jordan for his inspirational guidance and introducing me to ILT and IB and to Dr Colin Baxter-Jones for initiating our interest in TRT.

REFERENCES

Bagust, T.J. & Johnson, M.A. (1995) Avian infectious laryngotracheitis: virus-host interactions in relation to prospects for eradication. *Avian Pathology*, **24**: 373–391.

Banet-Noach, C., Simanov, L., Laham-Karam, N., Perk, S. & Bacharach, E. (2009) Longitudinal survey of avian metapneumoviruses in poultry in Israel: infiltration of field strains into vaccinated flocks. *Avian Diseases*, **53**: 184–189.

Bäyon-Auboyer, M.H., Arnauld, C., Toquin, D. & Eterradossi, N. (2000) Nucleotide sequences of the F, L and G protein genes of two non-A/non-B avian pneumoviruses (APV) reveal a novel APV subgroup. *Journal of General Virology*, **81**: 2723–2733.

Brandao, P.E., Sandri, T.L., Souza, S.P., Kuana, S.L., Richtzenhain, L.J. & Villarreal, L.Y.B. (2009) Recombination, point mutation and positive selection on the basis of molecular diversity of Brazilian strains of avian IBV, *VI International Symposium on Avian Corona- and Pneumoviruses and Complicating Pathogens (abstract)*, (Germany, Rauischholzhausen, University of Giessen).

Buys, S.B., du Preez, J.H. & Els, H.J. (1989) The isolation and attenuation of a virus causing rhinotracheitis in turkeys in South Africa. *Onderstepoort Journal of Veterinary Research*, **56**: 87–98.

Casais, R., Thiel, V., Siddell, S.G., Cavanagh, D. & Britton, P. (2001) Reverse genetics system for the avian coronavirus infectious bronchitis virus. *Journal of Virology*, **75**: 12359–12360.

Catelli, E., Cecchinato, M., Savage, C.E., Jones, R.C. & Naylor, C.J. (2006) Demonstration of loss of attenuation and extended field persistence of a live avian metapneumovirus vaccine. *Vaccine*, **30**: 6476–6482.

Cavanagh, D., Casais, R., Armesto, M., Hodgson, T., Izadkhasti, S., Davies, M., Lin, F., Tarpey, I. & Britton, P. (2007) Manipulation of the infectious bronchitis coronavirus genome for vaccine development and analysis of the accessory proteins. *Vaccine*, **26**: 5558–5562.

Cavanagh, D. & Gelb, J. (2008) Infectious bronchitis, in: Saif, Y.M. *et al.* (Eds) *Diseases of Poultry*, 12th ed., pp. 117–135 (Ames, IA, Blackwell Publishing).

Cecchinato, M., Catelli, E., Lupini, C., Richizzi, E., Clubbe, J. & Naylor, C. (2009) Evidence of AMPV attachment protein evolution coincident with mass live vaccine introduction in Italy (abstract), *VI International Symposium on Avian Corona- and Pneumoviruses and Complicating Pathogens*, (Germany, Rauischholzhausen, University of Giessen).

Cook, J.K. & Ellis, M.M. (1990) Attenuation of turkey rhinotracheitis virus by alternative passage in embryonated chicken eggs and tracheal organ cultures. *Avian Pathology*, **19**: 181–185.

Cook, J.K., Huggins, M.B., Woods, M.A., Orbell, S.J. & Mockett, A.P. (1995) Protection provided by a commercially available vaccine against different strains of turkey rhinotracheitis virus. *Veterinary Record*, **136**: 392–393.

Cook, J.K.A., Orbell, S.J., Woods, M.A. & Huggins, M.B. (1999) Breadth of protection of the respiratory tract provided by two different live attenuated infectious bronchitis vaccines against challenge with infectious bronchitis viruses of heterologous serotypes. *Avian Pathology*, **28**: 477–485.

Cook, J.K., Huggins, M.B., Orbell, S.J., Mawditt, K. & Cavanagh, D. (2001) Infectious bronchitis virus vaccine interferes with the replication of avian pneumovirus vaccine in domestic fowl. *Avian Pathology*, **30**: 233–242.

Davison, S., Gingerich, E.N., Casavant, S. & Eckroade, R.J. (2006) Evaluation of a live fowlpox-vectored infectious laryngotracheitis/avian encephalomyelitis vaccine against ILT viral challenge. *Avian Diseases*, **50**: 50–54.

Devlin, J.M., Browning, G.F., Gilkerson, J.R., Fenton, S.P. & Hartley, C.A. (2008) Comparison of the safety and protective efficacy of vaccination with glycoprotein-G deficient infectious laryngotracheitis virus delivered by eye drop, drinking water or aerosol. *Avian Pathology*, **37**: 83–88.

De Wit, J.J. & van de Sande, H. (2009) Efficacy of combined vaccines at day of hatching against D388 challenge in SPF and commercial chickens, *VI International Symposium on Avian Corona- and Pneumoviruses and Complicating Pathogens (abstract)*, (Germany, Rauischholzhausen, University of Giessen).

Dhinakar Raj, G. & Jones, R.C. (1996) Protectotypic differentiation of avian infectious bronchitis viruses using an in vitro challenge model. *Veterinary Microbiology*, **53**: 239–252.

Dhinakar Raj, G. & Jones, R.C. (1997*a*) Infectious bronchitis virus: immunopathogenesis of infection in the chicken. *Avian Pathology*, **26**: 677–706.

Dhinakar Raj, G. & Jones, R.C. (1997*b*) Effect of T-cell suppression by cyclosporin on primary and persistent infections of infectious bronchitis in chickens. *Avian Pathology*, **26**: 257–276.

Dolz, R., Pujols, J., Ordóñez, G., Porta, R. & Majó, N. (2006) Antigenic and molecular characterization of isolates of the Italy 02 infectious bronchitis virus genotype. *Avian Pathology*, **35**: 77–85.

Ducatez, M.F., Moreno Martin, A., Owoade, A.A., Olatoye, I.O., Alkali, B.R., Maikano, I., Snoeck, C.J., Sausy, A., Cordioli, P. & Muller, C.P. (2009) Characterization of a new genotype and serotype of infectious bronchitis virus in Western Africa. *Journal of General Virology* (forthcoming).

Ganapathy, K., Cargill, P., Montiel, E. & Jones, R.C. (2005) Interaction between live avian pneumovirus and Newcastle disease virus vaccines in specific pathogen free chickens. *Avian Pathology*, **34**: 297–302.

Gough, R.E. & Jones, R.C. (2008) Avian metapneumovirus, in: Saif, Y.M. *et al.* (Eds) *Diseases of Poultry*, 12th ed., pp. 101–110 (Ames, IA, Blackwell Publishing).

Gough, R.E., Collins, M.S. & Hancock, R.D. (1988) Isolation of turkey rhinotracheitis virus from five-day-old turkeys. *Veterinary Record*, **122**: 370–371.

Guy, S.J. & Garcia, M. (2008) Laryngotracheitis, in: Saif, Y.M. *et al.* (Eds) *Diseases of Poultry*, 12th ed., pp. 137–152 (Ames, IA, Blackwell Publishing).

Han, M.G. & Kim, S.J. (2003) Efficacy of live vaccines against infectious laryngotracheitis assessed by polymerase chain reaction-restriction fragment length polymorphism. *Avian Diseases*, **47**: 261–271.

Hughes, C.S., Gaskell, R.M., Jones, R.C., Bradbury, J.M. & Jordan, F.T.W. (1989) Effects of certain stress factors on the re-excretion of infectious laryngotracheitis virus from latently infected carrier birds. *Research in Veterinary Science*, **46**: 274–276.

Hughes, C.S., Williams, R.A., Gaskell, R.M., Jordan, F.T., Bradbury, J.M., Bennett, M. & Jones, R.C. (1991) Latency and reactivation of infectious laryngotracheitis vaccine virus. *Archives of Virology*, **121**: 213–218.

Hughes, L.A., Savage, C.E., Naylor, C.J., Bennett, M., Chantrey, J. & Jones, R.C. (2009) Genetically diverse coronaviruses in wild bird populations of northern England. *Emerging Infectious Diseases*, **15**: 1091–1095.

Jonassen, C.M., Kofstad, T., Larsen, I.L., Lovland, A., Handeland, K. & Follestad, A. (2005) Molecular identification and characterization of novel coronaviruses infecting graylag geese (*Anser anser*), feral pigeons (*Columbia livia*) and mallards (*Anas platyrhynchos*). *Journal of General Virology*, **86**: 1597–1607.

Jones, R.C. (1996) Avian pneumovirus infection: questions still unanswered. *Avian Pathology*, **25**: 639–648.

Jones, R.C. & Ambali, A.G. (1987) Re-excretion of an enterotropic infectious bronchitis virus by hens at point of lay after experimental infection at day old. *Veterinary Record*, **120**: 617–618.

Jones, R.C. & Jordan, F.T.W. (1972) The site of replication of replication of infectious bronchitis virus in the oviduct of experimentally infected hens. *Veterinary Record*, **89**: 317–318.

Jones, R.C., Williams, R.A., Baxter-Jones, C., Savage, C.E. & Wilding, G.P. (1988) Experimental infection of laying turkeys with rhinotracheitis virus: distribution of virus in tissues and serological response. *Avian Pathology*, **17**: 841–850.

Jones, R.C., Worthington, K.J., Capua, I. & Naylor, C.J. (2005) Efficacy of live infectious bronchitis vaccines against a novel European genotype Italy 02. *Veterinary Record*, **156**: 646–647.

Juhasz, K. & Easton, A.J. (1994) Extensive sequence variation in the attachment (G) protein gene of avian pneumovirus: evidence for two distinct subgroups. *Journal of General Virology*, **75**: 2873–2880.

Kirkpatrick, N.C., Mahmoudian, A., O'Rourke, D. & Noormohammadia, A.H. (2006) Differentiation of infectious laryngotracheitis virus isolates by restriction fragment length polymorphic analysis of polymerase chain reaction products amplified from multiple genes. *Avian Diseases*, **50**: 28–34.

Lee, E., Song, M.S., Shin, J.Y., Lee, Y.M., Kim, C.J., Lee, Y.S., Kim, H. & Choi, Y.K. (2007) Genetic characterization of avian metapneumovirus subtype C isolated from pheasants in a live bird market. *Virus Research*, **128**: 18–25.

Ling, R., Sinkovic, S., Toquin, D., Guionie, O., Eterradossi, N. & Easton, A.J. (2008) Deletion of the SH gene from avian metapneumovirus has a greater impact on virus production and immunogenicity in turkeys than deletion of the G gene or M2-2 open reading frame. *Journal of General Virology*, **89**: 525–533.

Liu, S., Chen, J., Chen, J., Kong, X., Shao, Y. & Han, Z. (2005) Isolation of avian infectious bronchitis coronavirus from domestic peafowl (*Pavo cristatus*) and teal (*Anas*). *Journal of General Virology*, **86**: 719–725.

Naylor, C.J., Brown, P.A., Edworthy, N., Ling, R., Jones, R.C., Savage, C.E. & Easton, A.J. (2004) Development of a reverse-genetics system for avian pneumovirus demonstrates that the small hydrophobic (SH) and attachment (G) genes are not essential for virus viability. *Journal of General Virology*, **85**: 3219–3227.

Neff, C., Sudler, C. & Hoop, R.K. (2008) Characterisation of western European field isolates of avian infectious laryngotracheitis virus by restriction fragment length polymorphism and sequencing. *Avian Diseases*, **52**: 278–283.

Ojkic, D., Swinton, J., Vallieres, M., Martin, E., Shapiro, J., Sanei, B. & Binnington, B. (2006) Characterisation of field isolates of infectious laryngotracheitis virus from Ontario. *Avian Pathology*, **35**: 286–292.

Oldoni, I., Rodríguez-Avila, A., Riblet, S. & García, M. (2008) Characterization of infectious laryngotracheitis virus (ILTV) isolates from commercial poultry by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). *Avian Diseases*, **52**: 59–63.

Oldoni, I., Rodríguez-Avila, A., Riblet, S., Zavala, G. & García, M. (2009) Pathogenicity and growth characteristics of selected infectious laryngotracheitis virus strains from the United States. *Avian Pathology*, **38**: 47–53.

Pohuang, T., Chansiripornchai, N., Tawatsin, A. & Sasipreeyajan, J. (2009) Detection and molecular characterization of infectious bronchitis virus isolated from recent outbreaks in broiler flocks in Thailand. *Journal of Veterinary Science*, **10**: 219–222.

Rimondi, A., Craig, M.I., Vagnozzi, A., König, G., Delamer, M. & Pereda, A. (2009) Molecular characterization of avian infectious bronchitis virus strains from outbreaks in Argentina (2001–2008). *Avian Pathology*, **38**: 149–153.

Rodríguez-Avila, A., Oldoni, I., Riblet, S. & Garcia, M. (2008) Evaluation of the protection elicited by direct and indirect exposure to live attenuated infectious laryngotracheitis virus vaccines against a recent challenge strain from the United States. *Avian Pathology*, **37**: 297–292.

Seal, B.S. (1998) Matrix protein gene nucleotide and predicted amino acid sequence demonstrate that the first U.S. avian pneumovirus isolate is distinct from European subtypes. *Virus Research*, **58**: 45–52.

Shin, H.J., Nagaraja, K.V., McComb, B., Halvorson, D.A., Jirjis, F.F., Shaw, D.P., Seal, B.S. & Njenga, M.K. (2002) Isolation of avian pneumovirus from mallard ducks that is genetically similar to viruses isolated from neighboring commercial turkeys. *Virus Research*, **26**: 207–212.

Sun, L., Zhang, G.H., Jiang, J.W., Fu, J.D., Ren, T. & Cao, W.S. (2007) A Massachusetts prototype-like coronavirus isolated from wild peafowls is pathogenic to chickens. *Virus Research*, **130**: 121–128.

Sun, H.L., Wang, Y.F., Tong, G.Z., Zhang, P.J., Miao, D.Y., Zhi, H.D., Wang, M. & Wang, M. (2008) Protection of chickens from Newcastle disease and infectious laryngotracheitis with a recombinant fowlpox virus expressing the F, HN genes of NDV and the gB gene of ILTV. *Avian Diseases*, **52**: 111–117.

Tan, B., Wang, H., Shang, L. & Yang, T. (2009) Co administration of chicken GM-CSF with a DNA vaccine expressing infectious bronchitis virus S1 glycoprotein enhances specific immune response and protects against IBV infection. *Archives of Virology*, **154**: 1117–1124.

Tarpey, I. & Huggins, M.B. (2007) Onset of immunity following in ovo delivery of avian metapneumovirus vaccines. *Veterinary Microbiology*, **20**: 134–139.

Tarpey, I., Orbell, S.J., Britton, P., Casais, R., Hodgson, T., Lin, F., Hogan, E. & Cavanagh, D. (2006) Safety and efficacy of an infectious bronchitis virus used for chicken embryo vaccination. *Vaccine*, **24**: 6830–6838.

Terregino, C., Toffan, A., Beato, M.S., De Nardi, R., Vascellari, M., Meini, A., Ortali, G., Mancin, M. & Capua, I. (2008) Pathogenicity of a QX strain of infectious bronchitis virus in specific pathogen free and commercial broiler chickens, and evaluation of protection induced by a vaccination programme based on the Ma5 and 4/91 serotypes. *Avian Pathology*, **37**: 487–493.

Tian, L., Wang, H.N., Lu, D., Zhang, Y.F., Wang, T. & Kang, R.M. (2008) The immunoreactivity of a chimeric multi-epitope DNA vaccine against IBV in chickens. *Biochemical and Biophysical Research Communications*, **377**: 221–225.

Toquin, D., Guionie, O., Jestin, V., Zwingelstein, F., Allee, C. & Eterradossi, N. (2006) European and American subgroup C isolates of avian metapneumovirus belong to different genetic lineages. *Virus Genes*, **32**: 97–103.

Turpin, E.A., Stallknecht, D.E., Slemons, R.D., Zsak, L. & Swayne, D.E. (2008) Evidence of avian metapneumovirus subtype C infection of wild birds in Georgia,

South Carolina, Arkansas and Ohio, USA. *Avian Pathology*, **37**: 343–351.

Villarreal, L.Y., Brandão, P.E., Chacón, J.L., Saidenberg, A.B., Assayag, M.S., Jones, R.C. & Ferreira, A.J. (2007*a*) Molecular characterization of infectious bronchitis virus strains isolated from the enteric contents of Brazilian laying hens and broilers. *Avian Diseases*, **51**: 974–978.

Villarreal, L.Y., Brandão, P.E., Chacón, J.L., Assayag, M.S., Maiorka, P.C., Raffi, P., Saidenberg, A.B., Jones, R.C. & Ferreira, A.J. (2007*b*) Orchitis in roosters with reduced fertility associated with avian infectious bronchitis virus and avian metapneumovirus infections. *Avian Diseases*, **51**: 900–905.

Villarreal, L.Y.B., Sandri, T.L., Assayag, L.J., Richtzenhain, L.J., Malo, A. & Brandao, P.E. (2009) Field observations after natural infection of Brazilian layer chickens with a phylogenetically divergent lineage of subtype B AMPV (abstract), *VI International Symposium on Avian Corona- and Pneumoviruses and Complicating Pathogens*, (Germany, Rauischholzhausen, University of Giessen).

Wang, Y.D., Wang, Y.L., Zhang, Z., Fan, G., Jiang, Y., Liu, X., Ding, J. & Wang, S. (1998) Isolation and identification of glandular stomach type IBV (QX IBV) in chickens. *Chinese Journal of Animal Quarantine*, **15**: 1–3.

Williams, R.A., Savage, C.E. & Jones, R.C. (1991) Development of a live attenuated vaccine against turkey rhinotracheitis. *Avian Pathology*, **20**: 45–55.

Williams, R.A., Al-Afaleq, A.I., Jordan, F.T.W., Bradbury, J.M., Gaskell, R.M., Bennett, M. & Jones, R.C. (1992) Pathogenicity of latent infectious laryngotracheitis in chickens. *Avian Pathology*, **21**: 287–294.

Williams, R.A., Bennett, M., Bradbury, J.M., Gaskell, R.M., Jones, R.C. & Jordan, F.T.W. (1993) Demonstration of sites of latency of infectious laryngotracheitis virus using the polymerase chain reaction. *Journal of General Virology*, **73**: 2415–242.

Woo, P.C., Lau, S.K., Lam, C.S., Lai, K.K., Huang, Y. & Lee, P. (2008) Comparative analysis of complete genome sequences of three avian coronaviruses reveals a novel group 3c coronavirus. *Journal of Virology*, **83**: 908–917.

Worthington, K.J., Sargent, B.A., Davelaar, F.G. & Jones, R.C. (2003) Immunity to avian pneumovirus infection in turkeys following in ovo vaccination with an attenuated vaccine. *Vaccine*, **28**: 1355–1362.

Worthington, K., Currie, R.E. & Jones, R.C. (2008) A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. *Avian Pathology*, **37**: 247–257.

Yang, T., Wang, H.N., Wang, X., Tang, J.N., Lu, D., Zhang, Y.F., Guo, Z.C., Li, Y.L., Gao, R. & Kang, R.M. (2009) The protective immune response against infectious bronchitis virus induced by multi-epitope based peptide vaccines. *Bioscience, Biotechnology and Biochemistry*, **73**: 1500–1504.

Zulperi, Z.M., Omar, A.R. & Arshad, S.S. (2009) Sequence and phylogenetic analysis of S1, S2, M, and N genes of infectious bronchitis virus isolates from Malaysia. *Virus Genes*, **38**: 383–391.