

# Respiratory viral diseases – lessons to be learned?

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**R**espiratory diseases caused by viruses continue to be a major cause of economic loss to the poultry sector. Losses are encountered in several ways, including mortalities, reduced weight gain, loss of egg production and quality, carcass downgrading, costs of vaccines, antimicrobial treatment for secondaries and diagnosis.

The most important virus diseases are those caused by Newcastle disease virus, influenza virus, infectious bronchitis virus, infectious laryngotracheitis and avian pneumovirus.

Other viruses may be associated with respiratory disease manifestations, such as poxviruses, adenoviruses and reoviruses, but these will not be covered. The purpose of this article is to look at what lessons we have learned about these diseases. In fact several lessons have been learned, from clinical experience, from careful experimental research and epidemiological observation.

One overriding lesson is that we are not good at completely eradicating these diseases. So, although most of them have been known for as long as the commercial poultry sector has been in existence, we still live with them, despite many years of research by outstanding scientists.

## Newcastle disease

This disease is worldwide and is probably the most important because of the severity of disease that some strains of virus can cause. Newcastle disease virus (NDV) is capable of infecting many species of birds including wild birds.

NDV has a single linear negative sense RNA genome coding for six proteins including the haemagglutinin-neuraminidase (HN) protein. Antibodies to this are the basis for haemagglutination-inhibition serology. NDV is less likely to mutate than AI and over the years has remained relatively stable. There is only one serotype of NDV which simplifies diagnosis and vaccination strategies.

NDV strains differ in their ability to cause disease and there are three pathotypes, the lentogenic viruses, which have low virulence and mesogenic and velogenic viruses which have medium and high virulence. The lentogenic NDV

strains cause a range of effects in poultry ranging from inapparent infection to mild respiratory disease and falls in egg production. Usually, mortality is low in uncomplicated cases, but disease can be more severe and prolonged where bacteria, mycoplasmas and perhaps other viruses are present.

In contrast to the lentogenic strains, the more virulent ones (mesogenic and velogenic) alone produce much more severe episodes which can have high mortality.

Disease may be systemic and several organs (respiratory, enteric, nervous etc.) are affected. The highly pathogenic NDV strains are on the Office International des Epizooties (OIE) A list of diseases.

Globally, ND is widespread and in 2001 it was reported in some 63 countries or territories. The highly virulent strains have a considerable negative impact on international trade.

The OIE states that they have 'potential for very serious and rapid spread irrespective of national borders which are of serious socio-economic or public health consequence and which are of major importance in the international trade of animals and animal products'.

While the lentogenic strains are not on list A, they can, nonetheless, have an impact on trade between countries. Most outbreaks of high virulence NDV have resulted from high virulence NDV circulating in poultry, although there is one reported example from Australia of a low virulence NDV mutating to a virulent state.

Diagnosis of ND of whatever virulence is strictly not possible without resorting to laboratory methods, since they may resemble diseases caused by other pathogens. Virus isolation is relatively easy in 9-10 day old fertile eggs and confirmation can be done by haemagglutination (HA) or haemagglutination inhibition (HI) with specific antiserum.

Other methods for diagnosis are immunofluorescence staining of tissues and the reverse transcriptase polymerase chain reaction (RT-PCR). HI and ELISA are used for serological surveillance.

The main approach to control of ND is to use vaccines and implementation of various biosecurity measures. Since there is only one serotype, the current empirically produced vaccines give good protection against NDV strains of whatever

level of virulence. However, as a general rule, vaccines with themselves a higher level of virulence (mesogenic) may be necessary against more virulent field strains. An important epidemiological aspect of ND is that the disease has a very wide host range and as many as 250 species of wild birds are capable of being infected and long distance migrators can play an important part in transmission.

This means that it is virtually impossible to keep infection out of a region and out of poultry unless they are kept in bird proof conditions.

The most recent outbreaks of ND in the UK were thought to have been caused by infected waterfowl flying further west than they would normally do. Recent work in the USA has shown that strains of NDV from birds such as doves after passage in chickens can become virulent for poultry.

This fact highlights the importance of biosecurity, which, in simple terms, means applying procedures that minimise or completely prevent the spread of infection. This is a recurring theme for all these diseases.

## Essential lessons learned from ND

NDV has a single serotype which is helpful for diagnosis and vaccine strategies. There are different levels of virulence and different levels of aggressive control may be necessary in different regions, depending on the strain.

NDV has a very wide avian host range and wild birds can be very important in spread of infection. Finally, good surveillance and biosecurity are essential.

## Avian influenza

Avian influenza (AI) is frequently generalised in terms of organs affected, but effects on the respiratory tract are a part of the disease so it is, therefore, included here. It is caused by a type A orthomyxovirus. AI viruses have eight segments of RNA, coding for 10 proteins, among which are two on the surface, the haemagglutinin (H) and the neuraminidase (N).

On the basis of the H and N, AI viruses are classified into 15H and 9N subtypes. AI viruses can readily undergo random

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mutations within gene segments as well as reassortment of segments between different AI viruses. Immunity is primarily against the specific H subtypes, so that there is no cross-protection between them and this complicates any vaccine formulations.

AI viruses are broadly divided into two groups according to the nature of the disease they cause – low pathogenicity (LPAI) or high pathogenicity (HPAI).

LPAI strains cause various problems ranging from clinically inapparent infections, to mild respiratory disease with loss of egg production.

Mortality rates are usually low. However, with HPAI, fatal systemic disease can be caused involving many organs in the body.

It is known that some LPAI H5 and H7 subtypes can mutate in the field to become HPAI. This was shown in several outbreaks, including Italy in 1999-2000 and in Chile in 2002. Such mutations have been shown to occur in the laboratory also.

HPAI can sometimes be important for human health. In 1997, there were six human deaths in the outbreak in Hong Kong with the H5N1 HPAI virus. In the recent outbreak in Holland (H7N7) there was one death, but there appeared to be predisposing factors. AI viruses can infect wild birds and they are an important means of introducing the virus into a new area.

Diagnosis of AI is done by growing the virus using fertile eggs and agar gel immunodiffusion, HI or ELISA to identify the H and N subtypes. PCR is becoming more widely used for this now.

Eradication of AI is the main thrust of control in most cases. Vaccination is sometimes used but it is essential to have a vaccine that matches the H and N subtypes of the infecting virus.

Once in an area, vaccination can be used for surrounding flocks, but generally, the appearance of AI is so unpredictable that the value of pre-emptive vaccination is questionable. In any case, vaccination may only reduce the load of virus shed rather than preventing it.

Good biosecurity is vital for AI control since the virus can be easily spread by movement of personnel and vehicles. AI viruses are excreted in the faeces in addition to via the respiratory tract.

In the recent outbreak in Holland, where the density of poultry populations is very high, there were 50 farms within one kilometre of the first outbreak and much of the spread in the locality was undoubtedly due to human movement.

### Essential lessons learned from AI

AI is caused by a virus with several subtypes and broadly two levels of pathogenicity.

The subtypes do not cross protect. Mutations can occur from time to time. This makes planned vaccination virtually impossible. Humans can occasionally become infected with the viruses and wild birds are an important part of transmission of infection. The main approach to control is eradication of affected flocks.

### Infectious bronchitis

Infectious bronchitis (IB) was first reported some 71 years ago. Despite intensive research, especially during the last 20 years, it is a disease which we live with and control by the use of empirically produced vaccines. IB has a relatively narrow host range (chicken and pheasant, but not, as far as we know, wild birds) but nonetheless is worldwide in distribution and likely to be present wherever chickens are kept in numbers. Its widespread nature is partly due to its highly infectious nature and rapid local spread, although we can only speculate as to how some strains are found in different continents while others are not.

There is some evidence of long term persistence in the chicken but the significance of this is unknown. IB is considered to be primarily a disease of the respiratory tract and it can be exacerbated by several other factors including other pathogens, although certain strains of the causal coronavirus (IBV) have a predilection for the kidneys and cause deaths in young birds. These nephropathogenic strains seem to be more localised and spread less than the conventional respiratory ones.

Eradication of IB at present seems improbable, if not impossible, but existing vaccines work well provided that they are administered accurately to each bird.

The biggest challenge to control strategies is the variation in the amino acid sequences of the S1 spike gene which leads to the irregular emergence of new variants, which are antigenically different to existing vaccine strains.

Most variants are unimportant and disappear, but occasionally a variant such as the recent one in Europe variously called 793B, 4/91 or CR88 appears which is pathogenic, persistent and sufficiently different antigenically to warrant a new vaccine.

Fortunately, not all variants need new vaccines and while laboratory cross-neutralisation tests have traditionally been a guide to in vivo interrelationships between IBV strains, we now know that it is how the bird vaccinated with a conventional vaccine perceives a novel strain, rather than its 'serotype'.

In other words, the protectotypic effect

is the more important than the serotypic or even genotypic relationships when it comes to establishing vaccine efficacy.

Considerable advances have been made in recent years in the understanding of the molecular structure of IBV strains and of immune mechanisms to the virus which will contribute significantly to control of the disease in the future.

For example, the recent production of a full length infectious clone of IBV should lead to safe, non-reverting designer vaccines for this disease, which will enable appropriate inserts to meet the challenge of new variants.

Work on immunity to different components of the virus has shown that immunity is not solely related to the S1 spike protein, but to other important components of the virus.

This, in addition to the fact that cell-mediated immunity plays an important role, helps to explain how some IB vaccines can be protective against apparently unrelated variants.

Molecular advances have also enabled the use of the polymerase chain reaction with RFLP or sequencing to take its place increasingly in diagnostic laboratories as the method of choice for identifying IBV strains in affected flocks.

This does not mean the demise of conventional isolation methods since securing the live virus is essential for pathogenesis studies and where required, new vaccine development.

Recent work has also shown that coronaviruses from enteritis in turkeys are related to IBV strains but are different. This is of particular interest, since a relatively overlooked aspect of IB in chickens is that many strains replicate in the intestine normally without causing pathological changes.

We have speculated that the propensity for IBV strains to mutate could, at some future date, lead to variants which cause disease in unexpected systems in the chicken such as the gut or liver – targets for coronaviruses for other species.

Perhaps then we should now consider whether the title 'infectious bronchitis viruses' is still appropriate.

Undoubtedly the body of information on IB and current and novel vaccines for the disease have attracted the attention of human virologists working on SARS.

### Essential lessons learned from IB

IBV has a relatively narrow host range compared with ND or AI, but is highly infectious. The virus can mutate, due to variation in the sequences of the amino acids in the S1 spike gene and this can lead to variants, against some of which existing vaccines are not protective.

Good surveillance is vital, including the

ability to detect new variants and molecular methods are now available to achieve this. Modern molecular methods offer hope of being able to carefully design IBV vaccines to meet the challenge of new variants. However, for the foreseeable future, we may always be one step behind in our attempts to control the disease.

### Infectious laryngotracheitis

ILT or LT is another 'old' disease (1925), principally of chickens but again also able to affect pheasants and sometimes, turkeys. However, there are some contrasts with IB. Although it is worldwide, it spreads less easily than IB. This leads to the situation where in some countries LT is endemic in certain areas, but absent from others.

The virus, a herpesvirus, has a single serotype, which simplifies vaccine strategies. Tissue distribution is restricted to the respiratory tract with regard to disease, although the causal herpesvirus becomes latent and persists in the trigeminal ganglia. From here, it can track back to the respiratory tissues and be re-excreted after stress, such as movement or sexual maturity. Such re-excreters may show no signs of disease and can act as an unrecognised source of infection. Live vaccines can similarly become latent and show similar re-excretion patterns. Indeed, it seems likely that in many instances, disease has been due to re-excreted vaccine virus, after latency, reverting to a virulent form.

In view of the important features of the virus and the disease outlined above, a persuasive case has been made for LT being a candidate for eradication, provided that the vaccines used were engineered to have a deletion, so that an appropriate ELISA could distinguish vaccinal antibodies from those due to field infection.

Molecular approaches have studied the LT herpesvirus in great detail, the holy grail being the development of a safe vaccine which does not go latent. Recently, the thymidine kinase gene has been shown to be responsible for virulence. In diagnosis, PCR has again been shown to be the most sensitive system for use and the addition of RFLP enables vaccine strains to be differentiated from field virus.

### Essential lessons learned from ILT

ILTV has a narrow host range and exists in only single serotype. Diagnosis and vaccine strategies have been relatively easy. Disease can range from subclinical to peracute. Latency of the virus is one of the main problems, since ILTV is a herpesvirus. Virus probably persists in naturally

infected or live vaccinated birds for life.

Given a marker vaccine, there are realistic prospects for eradication of ILT in certain regions.

### Avian pneumovirus infection

APV or more correctly avian metapneumoviruses, are the cause of turkey rhinotracheitis (TRT) and avian pneumovirus infection in chickens, which is sometimes followed by swollen head syndrome. TRT has been arguably, the most important disease of turkeys in the last 20 years.

Infection with these viruses was described only in the late 1970s in South Africa, so these are new diseases, relative to IB and LT. APV was subsequently described in Europe, Asia and South and Central America and it was shown initially that two subtypes of the virus (A and B) existed, based on differences in G (glycoprotein) genes.

It was not until 1996 that APV infection was described in the USA where the virus was found to differ in the M and F protein genes and has been called type C. Viruses isolated in France from ducks which are non-A, non-B have been called type D viruses.

The American virus, initially described in and eradicated from Colorado (hence 'C') is localised in Minnesota and surroundings, where there are huge populations of commercial turkeys. Australia and Canada remain free of infection. The type C virus is more closely related to the recently described human metapneumovirus than A or B.

Early work in Europe was centred on development of live and killed vaccines, the former after attenuation of field viruses. These generally are very effective provided that delivery is accurate.

The two subtypes each protect against both homologous and heterologous challenge and the differences are of greater significance for ELISA serology, where incorrect selection of antigen can lead to false negatives. TRT in turkeys is clearly an important respiratory pathogen, but the role of APV in chickens is less apparent.

While APV infection is exacerbated in chickens by *Mycoplasma gallisepticum*, *E. coli* and *Ornithobacterium rhinotracheale* (ORT), virulent IBV interferes with the replication of APV in the trachea and this has also been shown with commercial vaccines. This highlights the importance of timing of vaccine delivery.

In contrast, recent American work showed that virulent type C virus acts synergistically with virulent Newcastle disease virus in poults.

The timing of the use of live APV vaccines can be important. Use of live APV and IBV vaccines simultaneously shows that IBV inhibits the efficacy of APV vac-

cine and this phenomenon is being studied for other respiratory vaccines.

Recent evidence from Minnesota indicates that wild birds may play a part in the transmission of APV, although whether they develop disease is less clear. It may be that APVs have a very wide host range like Newcastle disease and influenza but this needs further investigation.

Alternative approaches to vaccination have been attempted for APV infections including vector vaccines and DNA vaccines. Another approach under investigation is the use of reverse genetics to produce an infectious clone.

As with other diseases, the intention is to devise a safe, replicating non-revertant vaccine but which will be able to compete in price with the existing ones.

Eradication of APV infection does seem a reality under some conditions, where flock density is low, the geography is favourable and biosecurity is high.

### Essential lessons learned from APV

While APV causes TRT, a very important disease of turkeys, its role in the chickens is less clear. Existing vaccines against subtypes A and B are effective but it is important to apply them correctly.

Subtypes A, B, C and non-A – non-B (D) are known; whether others will emerge is unknown. There is evidence of infection in wild birds but their role in spread is not yet clear.

### Summary of lessons learned

These viral respiratory diseases are hard to eradicate and we live with most of them, using vaccines for control.

The vaccines are generally effective when given properly. AI is controlled in most instances by eradication alone, although this is expensive.

In the case of IB, new variant viruses appear sporadically and some may warrant a change in vaccine strategy.

Devising effective vaccine programmes can be difficult, since some live vaccines may interfere with others if given simultaneously or close together.

All these respiratory diseases can be exacerbated by co-infection with other agents such as *E. coli*, ORT and mycoplasmas, so control of these other agents is very important. For ND, AI and perhaps APV infections, wild birds can be important in introducing infection into new regions.

For all these diseases good biosecurity should always be maintained, even when a new disease threat is not immediate. ■