Latest thinking on infectious bronchitis

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Infectious bronchitis (IB) is worldwide in distribution and is the most important endemic viral respiratory disease in countries where there is no Newcastle disease or avian influenza. It is caused by a coronavirus, infectious bronchitis virus (IBV) and affects chickens but not turkeys.

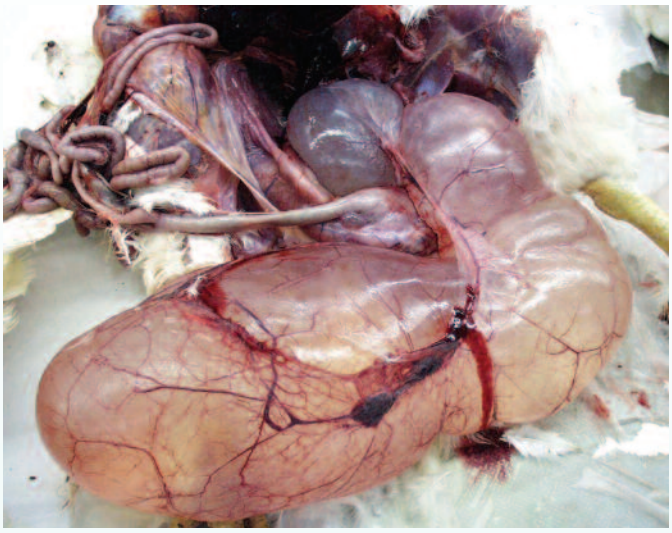
Although primarily a respiratory disease, in adult females IB causes significant losses in egg production and quality. Infection of female chicks soon after hatching with certain strains of virus can damage the oviduct so that at sexual maturity, oviducts develop abnormally, producing 'false layers'.

Some strains affect the kidneys causing deaths in young birds. Recently, there is some evidence that IBV may be implicated in other non-respiratory conditions of chickens.

Control is by the use of live and killed vaccines but the main problem is that the virus generates antigenic variants, through mutation or recombination of the gene that controls the important surface S1 spike protein.

Variants emerge from time to time which cannot be controlled by existing vaccines.

This article reviews infectious bronchitis and highlights the most recent thinking of several aspects of the virus and the disease.



Cystic oviduct of a 'false layer' after early infection with IBV variant. Field case (Hermann Block).

The disease

IB is recognised primarily as a respiratory disease of chickens of all ages which may be exacerbated and prolonged by intercurrent infection with other agents such as pathogenic E. coli or Mycoplasma gallisepticum.

The epithelium of the oviduct of the female is also highly susceptible to IBV strains and infection is critical at two stages of life.

The most familiar is of course, when the hen is in production, where IB can cause a delay in onset of lay or a serious fall in egg production and on return to lay, a high proportion of eggs laid will be of inferior quality. Production may never attain the expected level. The second crucial time for infection is in the days soon after hatching.

If female chicks become infected by certain genotypes of IBV to which they have no maternal antibodies, the virus can replicate in the epithelium of the oviduct.

This results in sufficient damage to the oviduct that when the bird reaches sexual maturity, regions of the organ may have developed abnormally or not at all, so the bird is unable to lay eggs externally.

Hens affected this way are called 'false layers'. This phenomenon has been seen recently with one of the new variants prevalent in Europe.

Some types of IBV are nephro-pathogenic and affect the kidneys so seriously that significant mortalities are seen in young birds.

Other disease manifestations observed recently which appear to have links with variant IBV types are enteritis and infertility in adult males. Proventriculitis has been observed in China.

The virus

IBV is a coronavirus, that is to say an RNA single stranded RNA virus. Particles are round to pleomorphic and the lipoprotein envelope is characterised by the presence of club-shaped spikes. These spikes are important in attachment to host cells and play a leading role in immunity to the virus.

There are numerous serotypes of the virus and the differences are based on variations in the S1 spike gene which has a hypervariable region, which enables variant types to be constantly emerging.

Molecular analysis of these differences is also used to differentiate types into genotypes.

Virus classification

The traditional method of classifying IBVs has been by cross-neutralisation tests, using specific antisera to neutralise viruses in fertile eggs or tracheal organ cultures.

This has led to the establishment of the well known serotypes including the (originally) American Massachusetts, Connecticut, Arkansas, DE072 and European D274, D1466, 793B types, etc.

More recently, molecular sequencing of specific genes has lead to a parallel classification, which is usually very similar. The most commonly examined gene is the S1 spike gene because this has a hypervariable region which is susceptible to variation, due to point mutations or recombination after infection with more than one virus.

Viruses that differ in the sequence of olignucleotides in this region of the spike gene are called genotypes, although the precise definition of this term has not been established for IBV.

Such variants are constantly emerging but are not equipped to survive. However, from time to time a variant emerges which is sufficiently different from existing vaccines that a new vaccine is required to control it.

This was the case in the early 1990s when a European variant called 793B or 4/91 appeared. The universally used Massachusetts vaccines offered no protection against this new virus, but now live vaccines for this variant (4/91, IB88) are used routinely in vaccine programmes.

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Fortunately, not all new variants require a new homologous vaccine. It has been found that combinations of two commonly used but serologically different live vaccines can give excellent protection against completely unrelated variants.

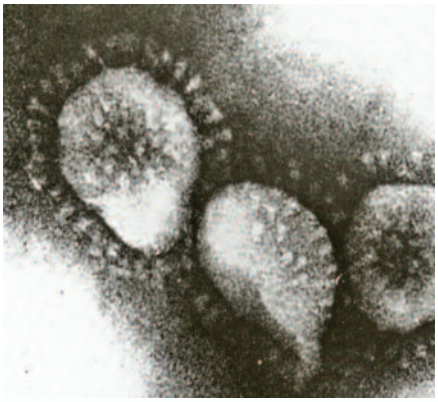
The mechanisms of this phenomenon have not been resolved but it is thought to relate to the broad immunity conferred by the two different vaccines, protection relating to non-spike proteins such as the membrane protein and cell mediated responses.

Determining if a current vaccine is efficacious against a variant relies ultimately on vaccination/challenge experiments in chickens. Merely comparing the S1 oligonucleotide sequences is insufficient to disclose whether a vaccine will be protective or not.

However, it has been reported that a shared sequence identity of less than 70% between a variant and a vaccine is likely to mean that the vaccine offers poor protection.

Only the chicken can determine if a vaccine is effective against a different virus and so in vivo trials are essential. Sometimes viruses are grouped according to the protective effects they confer as vaccines and this has lead to the term protectotype being used.

From the purely practical standpoint of control, this is the most important, albeit poorly defined, classification.



Electron micrograph of the infectious bronchitis coronavirus particles. Note the surface 'spikes'.

Hosts

Chickens of all ages are susceptible to the virus. Pheasants and turkeys may be infected by similar coronaviruses but they are different from IBV and the disease they cause is enteric.

Although coronaviruses have been detected in some species of wild birds, such as the graylag goose, widgeon, mallard and teal, they differ from infectious bronchitis virus at the molecular level, with the exception of one isolated from pancreatitis in pigeons which shared 99% identity in the S1 spike gene with a nephrotropic chicken strain from Shanghai and another from peafowl which is very closely related to the Massachusetts vaccine.

At the present stage of our knowledge, we cannot say that wild birds are influential in the long distance transmission of IBV in the way that they are for avian influenza viruses, although this aspect of IBV epidemiology has been poorly studied.

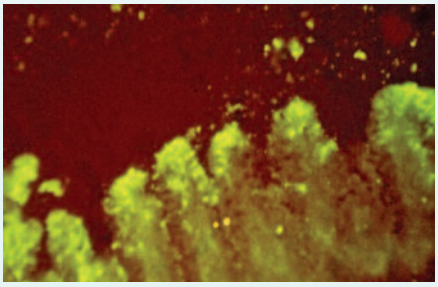
Global distribution

Some genotypes are ubiquitous and the best known is Massachusetts, probably because of its widespread use in vaccines.

Others, such as 793B, first described in Europe, appear to be widespread and antibodies have been found as far apart as Mexico and Thailand.

However, the USA is free from this type

Villi in the chicken intestine stained to show IBV in the cells (apple green) at the tips.



and has several indigenous IBVs which are not found elsewhere. Many laboratories in Eastern Asia, especially in China, have shown that there are several indigenous types in the region.

Australia, with its strict import controls has its own series of viruses, not found elsewhere. An interesting aspect of IBV in Australia is that over a 20 year period, it was found that strains have evolved from being predominantly nephrotropic to becoming respiratory pathogens.

Genotypes

A recent survey in Western Europe conducted at the University of Liverpool has indicated the appearance and prevalence of two 'new' genotypes, Italy 02 and QX. Both are widespread in this region.

The origin of Italy 02 is not known, but vaccine trials have shown that combinations of existing vaccines unrelated to this variant can be effective in controlling infection.

QX presents a very interesting situation, in that this virus was first described in China in 1996 and appeared in Europe in 2004, having traversed the Asian European land mass in only slightly less time than it took for avian influenza H5N1 to reach Europe from South East Asia.

Both these viruses cause respiratory disease but QX has been found to be responsible for losses associated with nephritis in young birds and false layers in mature hens.

Not unexpectedly, the survey demonstrated that the most prevalent types are those used as vaccines, namely, Massachusetts and 793B variants. Interestingly, Italy 02 has been the predominant 'wild type', for which there is no homologous vaccine.

Because of the frequent emergence of new variants, it is essential to have a constant surveillance system for IBVs. This enables the identification of prevalent genotypes and also alerts the industry to new viruses which may have the potential to become economically significant.

The reverse-transcriptase polymerase chain reaction (RT-PCR) method allied to S1 spike gene sequencing has proved to be ideal for this in its specificity and speed.

Chinese workers have identified many variants in recent years, including the QX type. So far this has been the only one identified in Europe. It will be interesting to see if further Chinese or other Asian viruses are transmitted over such long distances in the future, or if there is something biologically unusual about this one.

Diagnosis

Clinically, IB can be confused with diseases caused by other agents, most importantly, avian influenza and Newcastle disease and since no features of IB are pathognomonic, laboratory confirmation of the cause is essential.

If indeed IBV is the cause, then it is important to know the type of virus involved. In some laboratories, the traditional methods of virus isolation in fertile eggs or in tracheal organ cultures are used but are time consuming and expensive and the identity of the isolated virus is still required.

In many laboratories RT-PCR is now the method of choice followed by restriction fragment length polymorphism to identify known genotypes or by sequencing of the S1 spike gene, which enables a comparison with an international database of all reported IBV types. A recent report describes the use of a real-time PCR which is more sensitive than virus isolation, is very rapid and can quantitate amounts of virus in clinical samples. For antibody detection, there are no new developments, with ELISA useful as a broad screen and IBHI as a means of attempting to identify the type of the infecting virus.

One disadvantage with the serological tests for IB is that none of them correlate well with levels of immunity.

Control

The main approach to control of IB is by the use of live and killed vaccines. However, no consideration of control methods should be complete without close attention to biose-

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curity. It is worth emphasising that the programmes must be tailored to meet the local conditions, which include the perceived threat of infection and in particular, the identity of the infecting viruses. The latter in particular emphasises the need for good surveillance.

Generally, the available live and killed vaccines do a very good job of protecting against IB, provided that they are delivered accurately so that each bird received a full dose.

It should be remembered that not every new variant requires the development of a homologous vaccine – the so called 'fire-brigade' approach.

The use of combinations of existing vaccines derived from unrelated IBV genotypes has been shown to provide good broad immunity against a range of virus types unrelated to the vaccine viruses.

Future vaccines

While the current vaccines are generally very satisfactory, several laboratories have worked on the development of 'new generation' vaccines.

These have been derived by molecular methods, most of which are intended to enable vaccines to be tailored to particular local requirements and with the added advantage of not being able to revert to virulence.

Abnormal oviduct of a hen ('false layer') experimentally infected at day-old with IBV.

Vector vaccines have been exploited where the IBV spike gene is carried by a poxvirus or an adenovirus and DNA vaccines have also been developed.

Perhaps the most promising is one produced by the process of reverse genetics, whereby a DNA copy is made of the RNA genome which enables genes to be inserted or deleted.

For IBV, new appropriate spike genes can be included and an RNA copy of the virus is then re-constructed from the DNA. This vaccine has showed promise in preliminary trials.

The future

IB will continue to be a major cause of losses with IBV evolving and challenging vaccine protocols.

For the foreseeable future, it seems that existing empirically derived vaccines given alone or in combination will continue to provide the main means of control, while in the future, the new generation of vaccines need to prove their effectiveness but also need to be safe, affordable and easy to deliver.

With regard to disease, IBV evolution may mean that we will encounter more reports of these viruses being associated with non-IB conditions such as enteritis or male infertility.

It is also worth remembering that coronaviruses in other species may affect different organs and also occasionally change tropisms. ■

References are available from the author on request