Avian infectious bronchitis virus

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Summary
Infectious bronchitis virus (IBV) is prevalent in all countries with an intensive poultry industry, with the incidence of infection approaching 100% in most locations. Vaccination is only partially successful due to the continual emergence of antigenic variants. At many sites, multiple antigenic types are simultaneously present, requiring the application of multiple vaccines. Although many countries share some common antigenic types, IBV strains within a geographic region are unique and distinct, examples are Europe, the United States of America and Australia. Measures to restrict the introduction of exotic IBV strains should therefore be considered.

Infectious bronchitis has a significant economic impact; in broilers, production losses are due to poor weight gains, condemnation at processing and mortality, whilst in laying birds, losses are due to suboptimal egg production and downgrading of eggs.

Chickens and commercially reared pheasants are the only natural hosts for IBV. Other species are not considered as reservoirs of IBV. The majority of IBV strains cause tracheal lesions and respiratory disease with low mortality due to secondary bacterial infections, primarily in broilers. Nephropathogenic strains, in addition to tracheal lesions, also induce prominent kidney lesions with mortality of up to 25% in broilers. Strains of both pathotypes infect adult birds and affect egg production and egg quality to a variable degree.

Infected chicks are the major source of virus in the environment. Contaminated equipment and material are a potential source for indirect transmission over large distances. Virus is present in considerable titres in tracheal mucus and in faeces in the acute and recovery phases of disease, respectively. Virus spreads horizontally by aerosol (inhalation) or ingestion of faeces or contaminated feed or water. The virus is highly infectious. Clinical signs will develop in contact chicks within 36 h and in nearby sheds within one to two days. Infection is resolved within fourteen days with a rise in antibody titres. In a small number of chicks, latent infection is established with subsequent erratic shedding of virus for a prolonged period of time via both faeces and aerosol. Movement of live birds should be considered as a potential source for the introduction of IBV.

Isolation and identification of IBV is needed for positive diagnosis. The preferred method of isolation is to passage a sample in embryonating specified-pathogen-free chicken eggs. Identification is either by monoclonal antibody based enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction. Virus neutralisation test in tracheal organ culture is the best method for antigenic typing. Continual use of live vaccines complicates diagnosis since no simple diagnostic tool can differentiate a field from a vaccine strain. Nucleotide sequencing of the S1 glycoprotein is the only method to discriminate between all IBV strains.

Serology is also complicated by continual use of live vaccines. For surveillance purposes, ELISA is the method of choice, regardless of the antigenic type of IBV involved. The assay is used to monitor the response to vaccination, but field
Avian infectious bronchitis (IB) is primarily a respiratory infection of chickens. Nevertheless, three clinical manifestations of infectious bronchitis virus (IBV) infection will vary based on the age of chicks, pathogenicity of the virus strain and existing level of immunity. For detailed reviews of IBV, see Cavanagh and Naqui, and McMartin (14, 79).

Main presenting signs
Avian infectious bronchitis (IB) is primarily a respiratory infection of chickens. Nevertheless, three clinical manifestations of infectious bronchitis virus (IBV) infection are generally observed in the field, namely: respiratory disease, reproductive disorders and nephritis. Clinical signs will vary based on the age of chicks, pathogenicity of the virus strain and existing level of immunity. For detailed reviews of clinical signs associated with IB and pathology induced by IBV, see Cavanagh and Naqui, and McMartin (14, 79).

Respiratory disease
Respiratory disease is the most frequently observed syndrome caused by IBV (14, 48, 79). In broiler chicks of between two and six weeks of age, the main clinical signs seen are difficulty in breathing, tracheal rales, coughing and sneezing with or without nasal discharge. A generalised weakness is observed, accompanied by depression. Feed consumption and body weight are markedly reduced. Clinical signs in uncomplicated infections can be of short duration, commonly lasting less than seven days. Secondary infections due to 
*Escherichia coli* often follow, thereby accentuating the respiratory signs (19, 37). The chronic respiratory disease that develops may last for several weeks, with mortality between 5% and 25%. On necropsy, the trachea is congested with excessive amounts of mucus, and where infection has been complicated with *E. coli*, airsacculitis, pericarditis and perihepatitis may be observed (37). Similarly, the presence of *Mycoplasma* results in more severe clinical signs, with depressed growth and airsacculitis (17, 65).

Reproductive disorders
Infectious bronchitis virus infection at a young age and after maturity can both lead to reproductive problems in hens. In adult chickens clinical signs may not be present or may take the form of a mild respiratory disease with coughing, sneezing and rales which can go unnoticed unless the flock is examined carefully. A decline in egg production usually follows within seven to twelve days (78). The severity of the decline in egg production varies according to the stage of lay at infection and the strain of virus involved. Typically, these declines are between 3% and 10%, but reductions of up to 50% have also been observed. In some flocks, a decline in egg production will be the only feature. However, in many flocks the decline in egg production is also associated with eggs of smaller size and inferior shell and internal egg quality, seen as soft-, pale-shelled and misshapen eggs, and eggs with thin albumen (97). Flocks usually return to near normal production within one week, but occasionally six to eight weeks might elapse before a return to normal production. In many instances, production levels remain subnormal, usually 6% to 12% below pre-infection levels (7). On post-mortem examination, the oviduct length may be reduced and ovarian regression is noticed in some birds (97). If IBV infection occurs when chicks are less than two weeks of age, permanent damage of the oviduct may result, leading to poor laying capacity (26, 56).

Infectious bronchitis virus nephritis
The nephritic form of IB is characterised by mild and transient respiratory signs followed by depression, ruffled feathers, hunched stance, reluctance to move, excessive water intake, rapid weight loss and diarrhoea. Characteristically, wet litter is present. Death occurs four to five days after infection and ceases by day twelve after infection (27). On necropsy, carcasses are dehydrated and dark in colour, kidneys are enlarged and may be pale or marbled, and deposits of urates may be present in the ureters of some chicks.

Principal lesions
In chickens with respiratory disease, the main histological lesions are found in the trachea. However, these lesions are not pathognomonic for IBV. The virus replicates in ciliated epithelial cells causing deciliation, oedema, desquamation, hyperplasia and mononuclear cell infiltration of the submucosa (93). These changes are observed between three and nine days after infection. Regeneration of tracheal epithelium resumes approximately ten to fourteen days after infection.

In the oviduct, the height of the epithelial cells is reduced, and this is accompanied by a reduction or complete loss of cilia. The epithelial cells, especially the goblet cells, become cuboidal (97).

Following infection with nephropathogenic strains, virus replication first occurs in the trachea, causing histological lesions identical to those induced by respiratory strains. The route of spread to the kidney tissues is unknown. In the kidney, cytopathic changes become apparent, initially in the tubular epithelium. As a result, an interstitial inflammatory response is generated and polymorphonuclear leukocytes can
be seen, first in the medullary region and then in the cortex. Extensive tubular degradation and necrosis follow and these are prominent at between five and ten days after infection. Focal areas of uric acid precipitation appear in the kidney and may also accumulate in the ureters (92, 94). No correlation has been observed between the degree of kidney damage and the percentage of mortality observed in affected flocks (98). The reparative process commences in the kidneys approximately eleven days after infection.

Certain variant strains of IBV induce additional or differing pathology to that described above. Strain 793/B was associated with bilateral myopathy of the deep and superficial pectoral muscle in broiler breeders, previously unreported lesions in IBV infection (43). Strains of the Delaware 072 serotype were found to cause severe airsacculitis in the absence of secondary bacterial infection (S. Naqi, personal communication). Some strains of IBV are also reported to show particular affinity for, and persistence in, the proventriculus.

**Economic losses associated with the disease**

Flock management and the strain of virus involved play a major role in the impact of IBV infection. The principal losses are from production inefficiencies. The respiratory disease is debilitating, resulting in poor utilisation of feed by young chicks, and hence poor weight gains. Condemnation at processing due to airsacculitis also contributes to production losses. Following an outbreak of IB, an estimated 3% to 8% of the broilers can be condemned at the processing plant, in comparison to flocks in which IBV is controlled, where condemnation can be below 1%.

In layers and breeders, the main production losses are from non-realisation of full egg-laying potential. This may be a result of one of the following:  
- a) delayed maturity  
- b) decline in production during infection (estimated at between 3% and 50%)  
- c) sub-optimal production after recovery.

Additionally, losses are incurred due to downgrading of eggs. In breeders, the fertility rate could be reduced during and after an outbreak (7). In cases of IB nephritis, in addition to losses from poor weight gains and downgrading of carcasses, losses from mortality may be in the order of 10% to 25%.

McMartin estimates that an IBV infection in a commercial flock with the best possible management practice reduces income by approximately 3%, in comparison with a hypothetical flock free from IBV (79). The United States of America (USA) Animal Health Association Report (105) lists respiratory diseases as the most significant source of economic loss to the broiler industry, IBV being the major causative agent of respiratory infection in broilers (S. Naqi, personal communication).

**Incidence and distribution**

The disease was first reported in the early 1930s, and since then has been documented in all countries with an intensive poultry industry. Infectious bronchitis virus is thought to be endemic at the majority of poultry sites, with an incidence approaching 100%. Within a region where vaccination has not been practised, incidence of IBV infection was reported to be between 80% and 90% (48, 102, 103).

The importance of IB varies; in some areas the disease is considered as an on-going problem, whereas in others, periodic outbreaks occur which in some cases become epidemics. In colder areas, the incidence is usually higher in the winter months due to closing of the bird houses which compromises ventilation. Many episodes are caused by poor or inadequate vaccination. Where IB persists on a site, or in the case of an epidemic, the emergence or introduction of antigenic variants may be responsible.

**Morbidity and mortality**

In broiler flocks, morbidity is virtually 100%, whereas mortality is usually low. Early reports describe mortalities of 20% to 30%, and these were almost certainly due to mixed infections with other infectious agents such as E. coli or Mycoplasma. In younger chicks affected with IB nephritis, mortalities of up to 25% are common (28, 80).

**Description of the aetiological agent**

**Principal sites of replication**

The initial and principal site of viral replication is the epithelium of the trachea, where IBV can be detected at day one after infection. From this site, the virus spreads to other internal organs, such as the lung, spleen, liver, kidney, oviduct, ovaries, testes, digestive and intestinal tracts (31, 51, 74). The highest titre of virus, between $10^3$ and $10^4$ median egg infective doses, is found in the trachea and kidney tissues between three and five days post infection (51), and these tissues are the usual source for virus isolation in the acute phase of the disease.

**Strain variation**

**Pathotypic variation**

Strains of IBV differ in virulence or pathogenicity for the respiratory tract, kidney or oviduct. Although the virulence of many IBV strains has not been clearly defined, examples illustrate the predominant feature of each pathotype. The majority of IBV strains, including those of the Massachusetts (Mass) serotype, of which the M41 is the representative strain, produce prominent respiratory disease (14). Most of these strains do not induce mortality when acting alone. However, in experimental and field infections with E. coli, variable mortality rates are obtained, indicating the differing
pathogenic potential of strains to predispose chicks to the development of airsacculitis, pericarditis and perihepatitis (99).

Pathogenicity of strains of IBV for the oviduct varies greatly. Many, but not all strains of IBV are able to replicate in the epithelial cells of the oviduct, causing pathological changes (26). The majority of those that are able to replicate will induce a decline in egg production, while others will not (78). Some of the respiratory strains cause only a slight reduction in egg production, but markedly affect the colour of the egg shell (23).

Nephropathogenic strains have been a predominant IBV pathotype only in Australia (28), with sporadic isolation in other countries. However, during the last decade, nephropathogenic strains have emerged in many countries including Italy, the USA, Belgium, France, the People's Republic of China, Japan and Taipei China (13, 70, 106, 111). In some countries of Europe, these strains have become the predominant pathotype of concern (80). The nephropathogenic IBV strains are able to induce mortality, principally in chicks under the age of ten weeks (28), and differ markedly in virulence. The nephropathogenic strains of IBV isolated in Australia cause mortalities of between 5% and 80% in experimental infections (66; J. Ignjatovic, F.D. Ashton, R. Reece, P. Scott and P. Hooper, unpublished observation), whereas those found in other countries result in mortality between 5% and 50% (2, 81).

Antigenic variation
A multitude of IBV serotypes exist and this has become the major problem in diagnosis and control of IBV. Virus neutralisation (VN) or haemagglutination-inhibition (HI) tests are used to determine the IBV serotype. The VN test is the preferred method, as the HI test lacks some specificity (24). Strains of the Mass serotype have been the predominant antigenic type in many countries, the exceptions being Australia and New Zealand (Table I). Although strains of Mass serotype are still frequently isolated, many unique and new serotypes have emerged world-wide (Table I). Comparisons of nucleotide sequences of IBV isolates from different parts of the world indicate that strains from a particular geographic area are more closely related to each other than to strains from other distant regions (Fig. 1). With the exception of strains of the Mass serotype, IBV strains from Europe differ from those found in the USA or Australia, and each geographic group can be distinguished by the uniqueness of the genetic sequences.

Host range
Until recently, the chicken was considered as the only natural host of IBV. Chickens of all ages are susceptible, assuming that they have not had prior exposure to the virus and are not immune. Pheasants are the only other avian species that is now considered as a second natural host for IBV. Two reports from the United Kingdom (UK) have indicated recently that farm-reared pheasants are equally susceptible to IBV infection (44, 101). Clinically, the disease in pheasants resembled interstitial nephritis, with reduced egg production and high mortality. However, some species of pheasant are apparently not susceptible to IBV (5), or perhaps only certain strains of IBV are able to infect pheasants. Other avian species are not considered susceptible to infection with IBV. Antibodies to IBV were detected in turkeys, quails and in free-ranging rockhopper penguins (Eudyptes chrysolophus [chrysolophus])

Table I

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Fig. 1  
Phylogenetic relationship between strains of infectious bronchitis virus based on the nucleotide sequence of S1 glycoprotein available in GenBank

Sequences were aligned using the program ClustalW (104) and used to calculate a distance matrix from which the most likely phylogenetic tree was inferred using neighbour-joining method (62, 107), however, it is unclear whether IBV can be isolated from these species.

**In vitro propagation systems**

The most effective *in vitro* propagation system is embryonating chicken eggs that originate from a specified-pathogen-free (SPF) flock (87). Eggs of between nine and eleven days of incubation are inoculated by the allantoic route. Allantoic fluid collected 36 h to 48 h after inoculation contains a significant amount of virus, typically between $10^5$ and $10^7$ median egg infective doses. However, for IBV to grow in eggs to an appreciable titre, adaptation of the virus is necessary, usually requiring three to five passages. Egg-adapted IBV induces morphological changes in embryos, such as dwarfing and curling, enabling positive IBV diagnosis (14). If adaptation of IBV is not desired, the field samples should be propagated in tracheal organ cultures (21).

**Epidemiology**

**Sources and routes of infection**

Contact with infected chickens is the most likely source of infection, tracheo-bronchial exudate and faeces of these birds being the major sources of virus. The virus spreads horizontally by aerosol or ingestion. The rate of spread will depend on the virulence of virus and the immune status of the flock. The virus is highly infectious, and under natural conditions respiratory signs will develop in contact birds within 36 h. On a site, house-to-house spread will occur within one to two days, and between farms within three to four days. Faeces, and feed and drinking water that have been contaminated by faeces, are also sources of infection. The virus can survive for a considerable time in faeces and is suspected to represent a continuing source of re-infection in the recovery phase of the disease. Contaminated litter, footwear, clothing, utensils, equipment and personnel are all potential sources of virus for indirect transmission and have been implicated in IBV spread over large distances (36, 79, 95).

The role of vertical transmission in the epidemiology of IBV has not been clearly established. Vertical transmission of IBV has been suspected in one field case (76) and was demonstrated in another study in which experimentally infected hens layed infected eggs between one and six weeks after infection (20). Virus was also isolated from the day-old chicks of these hens. This suggests that IBV can be transmitted vertically. Infectious bronchitis virus was also recovered from the semen of cockerels for up to two weeks after inoculation (20), indicating the possibility that the oviduct of a susceptible hen, and also an egg in the oviduct, could be infected with such semen.

**Principal routes and duration of excretion**

During the acute phase of the disease, virus is copiously shed into the respiratory tract, and coughing birds shed virus into the environment. Infectious bronchitis virus can be recovered routinely from the trachea and lungs, between days one and seven post infection, in considerable titres. The virus can also be isolated from the cloacal contents from days one to twenty-four post infection. Faeces contain a significant amount of virus for a prolonged period of time – during an active infection, a recovery phase and during the course of chronic infections (3,19).

**Evidence of the carrier state or latency**

A true latency, such as that observed in herpesvirus infections, is not believed to occur in IBV infections. However, in some circumstances, IBV is thought to persist for a considerable time in infected birds at some privileged sites such as the kidney and the alimentary tract, particularly the caecal tonsils (4, 16, 35). Persistence and prolonged excretion of IBV in faeces by a small number of chicks between 49 and 227 days after infection has been demonstrated (4, 19). Re-excretion is suspected to be triggered by adverse environmental factors or changed physiological conditions. Chicks infected at one-day-old with enterotropic G strain, re-excreted virus at onset of lay into both the trachea and cloaca and continued to excrete the virus for a prolonged period (57). These chicks also re-excreted virus at twelve weeks following treatment with the immunosuppressive agent cyclosporin (6).
Persistently infected birds are considered to be the likely source of re-infection in layers which occurs at regular intervals (19). Persistence may be characteristic of some IBV strains only (3).

**Methods of spread**

Method of spread is airborne or mechanical transmission between birds, houses and farms. Airborne transmission is via aerosol and occurs readily between birds kept at a distance of over 1.5 m. Prevailing winds might also contribute to spread between farms that are separated by a distance of as much as 1,200 m (29). Mechanical transmission of virus is via personnel, material and equipment, and plays a role in transmission of IBV between flocks or farms. Movement of live birds, either as one-day-old chicks or as adult birds, should be considered as a potential source for the introduction of IBV. Although this has not been directly demonstrated, the strong experimental evidence of IBV persistence and re-excretion are an indication of the potential risk (3). Vectors do not appear to be a factor in the spread of IBV.

**Susceptibility of other species to infection**

Other species are not considered susceptible to natural infection with IBV. Infectious bronchitis virus can be propagated in suckling mice if inoculated by the intra-cerebral route (30). However, such virus passage appears to result in selection of a virus population which is not pathogenic for chicks (110). Suckling rabbits and guinea-pigs are also susceptible to intra-cerebral inoculation with IBV.

**Stability to environmental inactivation and disinfectants**

Infectious bronchitis virus is temperature sensitive; some strain differences may exist in terms of thermal sensitivity, but in general, most strains are inactivated at 56°C for 15 min to 45 min or at 45°C for 90 min (89). At room temperature, the virus will survive for only a few days. Viral infectivity is affected by storage at 4°C, and for long term storage, a temperature of −70°C is recommended. Lyophilised virus stored at 4°C will retain infectivity for at least twenty-one months (50). Infectious bronchitis virus is easily inactivated by many common disinfectants including 70% ethanol, chloroform, 1% phenol, 1% formalin and iodine (58). The virus is more stable at a low pH than at a high pH. At pH 3.0, the virus is stable for fourteen days. During vaccine administration, IBV longevity is affected by water quality and contents or additives (58). The virus appears to be able to survive for a considerable period of time in litter containing faeces.

**Risks of importing the disease through poultry and poultry products**

Infectious bronchitis is a List B notifiable disease, and countries importing poultry genetic material should require testing to be performed (88). Hatching eggs do not represent a risk if the flock of origin is antibody-free. Day-old chicks and older birds that are antibody-negative and have not been vaccinated prior to despatch are also low risk. However, these birds must be kept in quarantine conditions for at least four weeks, and for up to fifty days after arrival at a new location (88). Live birds of all ages that have been vaccinated and are antibody-positive, represent a risk since a small percentage of such birds may excrete virus until at least twenty weeks after infection. Processed poultry meat which has undergone treatment at temperatures above 56°C for 30 min to 45 min and is destined for human consumption is also considered a low risk. Infectious bronchitis virus has not been implicated as an accidental vaccine contaminant.

**Measures to reduce the risk of infectious bronchitis virus spread through trade**

Controlled studies regarding the possibility of IBV transmission through trade in poultry products have not been performed. There is circumstantial evidence however, which indicates that unrestricted trade might have contributed to the spread of some IBV types. In the most rigorous analysis, and if the precautionary principle is adopted, eggs and live birds should be considered as potential sources and carriers of IBV. However, current trade practices have accepted that the risk of spread of IBV through trade in eggs and live chicks is low. Eggs are disinfected after collection as is required for the control of other pathogens. Chicks that are destined for export are hatched on premises with an acceptable or approved level of biosecurity. Whether vaccination of chicks against IBV will be practiced at the hatchery will be determined between trading partners or may be regulated by the importing country. Countries importing eggs for hatching or live chicks might wish to quarantine the flock for 3 to 4 weeks in the presence of local antibody-free sentinel chicks.

**Consequences of introduction into a susceptible population**

Although IBV is distributed world-wide, strong consideration should be given to measures to restrict the introduction of exotic IBV variants. As outlined, many countries share common IBV serotypes (Table I). However, in many of these countries or regions, new economically important strains have arisen that require different vaccines for control. An inclusion of an unrelated IBV will necessitate the introduction of new vaccines, which will ultimately be reflected in higher costs, particularly in management, since a greater number of vaccines will need to be administered. In addition to the immediate economic significance, introduction of an exotic strain increases the pool of genetically different viruses that circulate on a site. This increases the likelihood of generating new and more variable strains through processes such as recombination (68). In both the USA and Australia, although variants continue to emerge, IBV strains have remained specific for each region (Fig. 1), illustrating the benefits of quarantine.
Diagnostic methods

Positive diagnosis of IB cannot be made on clinical signs alone, since other avian pathogens such as Newcastle disease, infectious laryngotracheitis and avian pneumoviruses can produce similar clinical signs. Involvement of IBV should be confirmed by virus isolation and, if desired, the antigenic type can be determined by VN. Serological tests to document a recent IBV infection are only valid if no prior flock exposure to IBV has occurred, or if the antibody status of the flock is monitored and enables unambiguous detection of a specific rise in IBV antibody titres.

Laboratory tests to confirm diagnosis

A number of tests are available (87); those considered most useful are discussed below. In adoption of any of the tests, consideration should be given to the diagnostic capability of laboratories involved and to the purpose of the positive IBV identification. None of the existing in vitro diagnostic tests will provide all of the necessary information. Only in vivo cross-protection experiments will demonstrate if the vaccine used on the premises is effective against the isolated field strain.

Virus isolation

The most common diagnostic method is to passage samples in embryonating SPF chicken eggs. The test is lengthy since three to five passages are required for a field isolate to become adapted to the egg and cause gross embryo pathology typical of IBV. A sample is classified as negative if no embryo changes are induced at the sixth egg passage. Allantoic fluid of the third to sixth passages usually contains enough virus to be confirmed by an antigen-detecting ELISA or electron microscopy, if such a facility is available. Fluorescent staining of amniotic cells using reference IBV sera may be used to confirm virus isolation (18). Tracheal organ culture is the most sensitive method for virus isolation and serotyping, particularly if adaptation of virus is not desired, however a specialised laboratory is required (21). Propagation of virus can also be performed in chicken embryo kidney cells, although this method has been used only on material already propagated in eggs, and some selection of a particular virus population is likely to occur during this process (75).

Antigen-detecting enzyme-linked immunosorbent assay

Antigen-detecting ELISA is the most rapid test to confirm the presence of IBV, whether in a field sample or in allantoic fluid (53, 61, 67, 85, 86). All antigenic types can be detected, however commercial kits are not widely available and diagnostic laboratories may need to source the reagents from the laboratories in which the monoclonal antibodies have been produced. An antigen-detecting ELISA kit and monoclonal antibodies developed in Australia are available commercially.

Reverse transcriptase polymerase chain reaction

Reverse transcriptase polymerase chain reaction (RT-PCR) is a reliable and sensitive method which can be used to test field samples. The test can be tailored to detect all IBV strains by using primers for the conserved N protein or S2 glycoprotein of IBV (1, 112). However, RT-PCR is complex to perform and requires a level of expertise that may not be available in a diagnostic laboratory.

Strain differentiation

No easy method exists to differentiate between various IBV strains, due to the existence of multiple serotypes. Any of the following methods can be used, but each has some disadvantages. The most accurate strain differentiation is achieved by nucleotide sequencing of the S1 glycoprotein, however this cannot be done on a routine basis. Serotyping using VN in tracheal organ cultures or embryonating eggs is the recommended method (47, 55). The VN test is lengthy and expensive, since multiple sera and IBV strains must be used in a two-way neutralisation assay (87). Strains of Mass, Connecticut (Conn), Arkansas (Ark) or D207 serotypes, can be differentiated by monoclonal antibodies in ELISA (60, 67, 86). However, isolation of an IBV strain that reacts with a serotype-specific monoclonal antibody does not always guarantee that the isolate is identical to the expected serotype. Some of the recently developed methods based on RT-PCR aim to differentiate certain serotypes (63, 69, 72). Further developments in this area, particularly RT-PCR followed by restriction fragment analysis may provide more user-friendly, comprehensive and rapid differentiation methods.

Diagnosis is complicated by the simultaneous presence of vaccine and field strains, which is suspected to occur in many field samples (15). No simple diagnostic tools are available to distinguish between strains of IBV in the same sample. Some molecular probes enable differentiation of strains simultaneously present in the sample (15, 45). However, these probes will only detect IBV genotypes that have been recognised previously.

Specificity of tests for surveillance and imports

For surveillance purposes, an antibody-detecting ELISA is the most appropriate test. A number of commercial kits are available which will all detect seroconversion to IBV regardless of the antigenic type or origin of the virus. In an antibody negative flock that has been exposed to IBV, ELISA antibodies will appear approximately ten days after infection, and will continue to rise until approximately four weeks after infection, at which time the level of antibodies will stabilise (46). An ELISA will detect an antibody response in broilers following vaccination at one day of age, and also in layers and breeders vaccinated at three weeks of age and re-vaccinated at twelve to eighteen weeks of age (83, 109).

Imported poultry that are serologically negative should be quarantined for at least twenty-one days in the presence of sentinel SPF chicks. Imported chicks should be tested for
antibody levels at the time of import and twenty-one days later. Chicks which have been recently infected will give a negative result at the first testing but will seroconvert during the observation period and become antibody positive twenty-one days later. Sentinels will also seroconvert, confirming in-contact transmission. Chicks which are immune at the time of import should be quarantined for a period of at least seven to eight weeks, preferably longer, in the presence of sentinel SPF chicks. If imported poultry is persistently infected, the antibody level will remain unchanged during the observation period. However, if the birds are excreting IBV, sentinel chicks will seroconvert.

If chicks are immune at the time of import and are persistently infected, a quarantine period of seven to eight weeks may not be sufficient, since some of the persistently infected birds may re-excrete virus only under certain conditions, for example at commencement of lay.

Public health implications

Risk to public health

No risks to human health are suspected or have been demonstrated to arise from IBV. Neutralising antibodies have been detected in people working with commercial chicken flocks (82), but the significance of this remains unknown.

Possibility of transmission from human to human or human to animal

No evidence exists to suggest that humans act as a reservoir for active replication of IBV and no evidence has been found of transmission from human to human, or human to animal. Humans can only transmit IBV to chicks by mechanical means. Reported isolation of an ‘avian IBV-like’ virus from humans was later demonstrated to be an isolate of human coronavirus and not an IBV coronavirus (59).

Methods of prevention and control

Exclusion and eradication

None of the countries which have an intensive poultry industry are free from IBV. Although attempts have been made, at the regional level, to keep flocks free from IBV, none have been successful. Given the highly infectious nature of the virus, even the strictest preventative measures are sometimes not sufficient. In one case, flocks well separated from other commercial farms became infected despite strict attention to quarantine (95). In another case, all flocks in a region became infected by a variant IBV strain from another region although import regulations prohibited the entry of live poultry and eggs (79). Only the strictest of quarantine measures, such as those used to maintain SPF poultry, and which cannot be justified commercially, appear to be able to exclude IBV. Under normal flock management with ‘all-in/all-out’ operations, cleaning and disinfection between batches will limit the level of infection to a minimum; however, exclusion of IBV has not been achieved through such measures. Under the present circumstances, eradication is not a consideration, but may become a long-term prospect with the introduction of recombinant IBV vaccines, which would replace live vaccines, and thus reduce the levels of infectious virus in the environment.

Breeding to increase resistance

Breeding to increase resistance to IBV is not practised. As evident from both field and experimental studies, all chicks are susceptible to infection with IBV. However, the outcome of the infection may vary in different lines. In experimental studies that included inbred laboratory lines, varying mortality rates were observed in lines inoculated with a pool of respiratory IBV strains and E. coli (11, 12, 99). Chicks of one ‘susceptible’ line showed more severe respiratory signs and had a prolonged recovery phase in comparison to chicks of a ‘resistant’ line. However, no differences were found between these ‘susceptible’ and ‘resistant’ lines in the rate of virus replication (90). Similar genetic differences between inbred lines have also been observed following infection with a nephropathogenic strain; one line exhibited high mortality from nephritis whereas no mortality occurred in another line (J. Ignjatovic, R. Reece and F.D. Ashton, unpublished observation). Another study noted that White Leghorns were severely affected by IB nephritis in comparison to a layer line from Australia which exhibited only mild clinical disease (27).

Vaccination

The only practical means of controlling IB is vaccination, which is routinely used throughout the intensive poultry industry. The following factors are a feature of IB vaccination:

a) vaccinal immunity is not long-lasting and re-vaccination is necessary

b) the selection of an appropriate antigenic type for the region is important, given the existence of wide antigenic variation

c) timing and method of vaccine application will vary for different flocks and may require adjustment according to practical experiences.

Type of vaccine used and effectiveness in protecting against infection and disease

Live and inactivated vaccines are used. Vaccination programmes and procedures may differ from one country to another, or even from one farm to another within the same country, depending on local conditions.

Live vaccines

Live vaccines are in widespread use. These vaccines represent IBV strains that have been passaged in embryonated chicken eggs to achieve a reduction in virulence for the respiratory
Inactivated vaccines provide high and uniform levels of antibodies that persist for longer periods than those induced by live vaccination (8, 9, 42). These high levels of antibodies are particularly useful in providing protection for the internal organs by preventing spread of the virus. In layers and breeders, inactivated vaccines provide protection against reductions in egg production, which might not always be afforded by live vaccination (8). In addition, in breeders, progeny chicks will be protected by maternally transferred antibodies. Progeny chicks that originate from breeders vaccinated with inactivated vaccines have high and uniform maternal antibody levels in comparison to breeders from dams vaccinated with live vaccines only. Most of the inactivated vaccines are of one type, Mass M41; however, bivalent vaccines that incorporate additional variant antigens may also be necessary (38, 84). Inactivated vaccines are produced from IBV-infected allantoic fluid, which is inactivated and usually formulated as oil emulsion vaccine. The disadvantage of such vaccines is the expense.

Possible reasons for vaccine failures

A number of factors influence the outcome of live vaccination (77). Vaccine preparations should be appropriately stored at all times and not used after the expiry date. The efficacy of vaccines will decline if stored for long periods at room temperature and if diluted in an inappropriate solution. Vaccine failure may also result if the chosen vaccine is not protecting against the prevalent antigenic type; if the vaccine is too mild or too virulent; or if the vaccinating dose per chick is too low due to excessive dilution of the vaccine. The route of vaccine application will affect the level and the duration of immunity as well as the vaccine reaction. The chosen route will depend upon previous experience or on the degree of clinical reaction in the flock. Manufacturers state the preferred method of vaccine application which can vary according to the strain.

Inactivated vaccines, if applied correctly, perform well in most situations. The lack of efficacy of inactivated vaccines may be due to inappropriate priming or because challenge strains are of a variant serotype which is not included in the common inactivated IB vaccines.

Ability to distinguish between vaccine and field strains and between immune responses to these strains

No reagents or simple molecular markers are able to distinguish a vaccine from a field strain. The simple procedure is to determine whether a field sample causes prominent dwarfing and pathology in embryonating eggs in the first inoculation. If so, the sample is likely to be a vaccine strain. The result needs to be confirmed by other tests such as VN and nucleotide sequencing. Certain IBV vaccines, such as Mass, Conn, Ark or D274 can be differentiated by serotype-specific monoclonal antibodies (67, 86). Nucleotide sequencing of the S1 gene can differentiate all vaccine strains and is the method of choice. No simple serological methods
are able to differentiate between exposure of commercial flocks to different IBV serotypes. A VN test can be used (25), but considerable cross-reaction can occur (40), and extensive testing is required involving IBV strains of many serotypes. Similarly, the use of an HI test is restricted, since exposure to multiple field strains of IBV can mask the detection of serotype-specific antibodies (10).

Virus de la bronchite infectieuse aviaire
J. Ignjatovic & S. Sapats

Résumé
Le virus de la bronchite infectieuse est présent dans tous les pays pratiquant une aviculture industrielle intensive, l’incidence étant souvent proche de 100 %. La vaccination ne donne pas des résultats pleinement satisfaisants en raison de l’apparition permanente de variants antigéniques. Dans de nombreux élevages, des types antigéniques multiples sont présents simultanément, d’où la nécessité de recourir à des vaccins multiples. Nombre de pays ont en commun certains types antigéniques, mais les souches du virus de la bronchite infectieuse au sein d’une même zone géographique sont uniques et distinctes ; c’est notamment le cas de l’Europe, des États-Unis d’Amérique et de l’Australie. Il convient donc d’envisager des mesures visant à restreindre l’introduction de souches exotiques de ce virus.

Cette maladie a d’importantes conséquences économiques : chez les poulets de chair, les pertes sont dues aux retards de croissance, aux saisies à l’abattoir et à la mortalité, alors que chez les pondeuses, elles sont liées à une perturbation de la ponte et à un déclassement des œufs. Les poulets et faisans d’élevage sont les seuls hôtes naturels du virus de la bronchite infectieuse. Les autres espèces ne sont pas considérées comme des réservoirs de ce virus. La plupart des souches du virus provoquent des lésions de la trachée et des maladies respiratoires avec une légère mortalité due à des surinfections bactériennes, essentiellement chez les poulets de chair. Certaines souches néphropathogéniques occasionnent, outre des lésions de la trachée, des lésions rénales, la mortalité pouvant alors s’élèver à 25 % chez les poulets de chair. Ces deux types de souches infectent les sujets adultes et affectent à des degrés divers la ponte et la qualité des œufs.

Les volailles infectées sont la principale source du virus dans l’environnement. Les installations et le matériel contaminés représentent une source potentielle de transmission indirecte sur de longues distances. Le virus est présent à des titres très élevés dans la muqueuse trachéale lors des phases aiguës de la maladie, ainsi que dans les déjections en période de guérison. Le virus se propage horizontalement par aérosols (inhalation) ou ingestion de déjections, d’aliments ou d’eau contaminés. Son pouvoir infectieux est élevé. Les signes cliniques apparaissent chez les poussins 36 heures après leur contact avec des sujets atteints ; dans les bâtiments voisins, l’infection apparaît dans un délai d’un à deux jours. La guérison intervient au bout de 14 jours, avec l’élévation des titres d’anticorps. Chez un petit nombre de poussins, on observe une infection latente avec élimination du virus pendant une longue période, dans les déjections et les aérosols. Les déplacements de volailles vivantes doivent être considérés comme une source potentielle d’introduction du virus de la bronchite infectieuse. Les œufs et les poussins provenant d’élevages reconnus indemnes sont les seuls à ne présenter aucun risque.

Le diagnostic doit être établi par l’isolement et l’identification du virus. La méthode d’isolement privilégiée est celle de l’inoculation d’œufs de poule embryonnés.
exempts d’organismes pathogènes spécifiques. Le virus est identifié soit par une épreuve immuno-enzymatique (ELISA) utilisant des anticorps monoclonaux, soit par l’amplification en chaîne par polymérase. La neutralisation virale dans une culture réalisée à partir d’écouvillons trachéaux est la meilleure méthode de typage antigénique. L’utilisation permanente de vaccins à virus vivant complique le diagnostic car il n’existe pas d’outil diagnostique simple permettant de distinguer les souches sauvages des souches vaccinales. Le séquençage des nucléotides de la glycoprotéine S1 est la seule technique permettant de distinguer les différentes souches du virus de la bronchite infectieuse.

L’utilisation permanente de vaccins à virus vivant complique également les examens sérologiques. Pour les besoins de l’épidémio-surveillance, l’épreuve ELISA est la plus indiquée, quel que soit le type antigénique du virus concerné. L’épreuve est utilisée pour suivre la réponse à la vaccination, mais l’apparition d’une infection naturelle ne peut être décelée que si le statut immunologique de l’élevage est contrôlé régulièrement. Le type antigénique de la souche responsable ne peut être identifié par l’épreuve ELISA.

**Mots-clés**


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**El virus de la bronquitis infecciosa aviar**

J. Ignjatović & S. Sapats

**Resumen**

El virus de la bronquitis infecciosa aviar es prevalente en todos los países que albergan explotaciones avícolas de tipo intensivo, hasta el punto de que en muchas áreas la incidencia de la infección ronda el 100%. Debido a la continua aparición de variantes antigénicas, las vacunaciones resultan eficaces sólo en parte. En muchas explotaciones están presentes diversos tipos antigénicos a la vez, lo que requiere la aplicación simultánea de varias vacunas. Aunque hay algunos tipos antigénicos comunes a muchos países, las cepas víricas presentes en cada región geográfica revisten un carácter único y distintivo (valgan, entre otros ejemplos, los de Europa, Estados Unidos de América o Australia). Por ello sería necesario estudiar la aplicación de medidas capaces de contener la penetración de cepas exóticas.

La bronquitis infecciosa da lugar a considerables perjuicios económicos: en pollos asaderos, las pérdidas productivas están ligadas al escaso aumento de peso, el descarte de ejemplares no aptos para el consumo, y a la tasa de mortalidad; en aves ponedoras, las pérdidas derivan de la menor producción de huevos y de su inferior calidad.

Los únicos huéspedes naturales de este virus son los pollos y los faisanes criados a escala industrial. Las demás especies no están consideradas como reservorios víricos. La mayoría de las cepas del virus provocan lesiones en la tráquea y la subsiguiente enfermedad respiratoria, con cierta mortalidad, aunque escasa, ligada a infecciones bacterianas secundarias, principalmente en pollos asaderos. Además de las lesiones traqueales, las cepas nefropatogénicas provocan importantes lesiones renales y dan lugar a una tasa de mortalidad de hasta el 25% en pollos asaderos. Las aves adultas se ven afectadas por cepas de ambos tipos patogénicos, con consecuencias variables en cuanto a la producción y la calidad de los huevos.
Los pollitos infectados constituyen la principal fuente excretora del virus al entorno. El material o los productos contaminados son un posible foco de transmisión indirecta a larga distancia. En las fases aguda y de convalecencia, el virus está presente a títulos considerables en el mucus traqueal y las heces, respectivamente. La infección se transmite horizontalmente, ya sea por aerosol (inhalación) o por ingestión de heces o agua y alimentos contaminados. Se trata de un virus extremadamente infeccioso: los pollitos expuestos a la fuente del virus manifestarán signos clínicos en un plazo de 36 horas, y los ejemplares de corrales adyacentes lo harán al cabo de uno a dos días. La infección remite dentro de los 14 días siguientes, con un aumento del título de anticuerpos, aunque unos pocos pollitos la conservarán en forma latente y seguirán excretando el virus erráticamente durante largo tiempo, por vía tanto fecal como respiratoria. Por ello debe considerarse que el movimiento de ejemplares vivos constituye un posible mecanismo de introducción del virus de la bronquitis infecciosa aviar. Sólo serán inocuos a ciencia cierta los huevos y pollitos procedentes de bandadas seronegativas.

Para el diagnóstico de confirmación es necesario aislar e identificar el virus. El mejor método de aislamiento consiste en inocular una muestra dentro de huevos embrionarios de pollo libres del patógeno especificado. Para la identificación puede usarse indistintamente un ensayo inmunoenzimático (ELISA) con anticuerpos monoclonales o la amplificación en cadena por la polimerasa (PCR). La prueba de neutralización viral sobre un cultivo de tejido traqueal, por su parte, es el mejor método de tipificación antigénica. El empleo incesante de vacunas vivas complica el diagnóstico, pues no hay ninguna técnica de diagnóstico sencilla capaz de distinguir entre cepas salvajes y cepas de vacuna. El único método que permite discriminar entre todas las cepas del virus es la secuenciación de la glicoproteína S1.

El uso continuo de vacunas vivas dificulta también el uso de técnicas serológicas. Para fines de vigilancia, y con independencia del tipo antigénico del que se trate, el método más indicado es el ELISA. Esta técnica se utiliza para controlar la respuesta a la vacunación, pero para detectar una infección de campo es preciso hacer un seguimiento continuo de los niveles de anticuerpos que presenta la bandada. La técnica ELISA no permite determinar el tipo antigénico de la cepa causante de una infección.

**Palabras clave**
Bronquitis infecciosa aviar — Comercio internacional — Enfermedades aviares — Epidemiología — Fuente de infección — Transmisión — Virus de la bronquitis infecciosa aviar.

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