Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER

28

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Coronaviridae

The family *Coronaviridae* is a member of the order *Nidovirales*, the only other family in the order being the *Arteriviridae*. These are linear, single-stranded RNA viruses of positive sense. The *Coronaviridae* family is divided into two genera, *Coronavirus* and *Torovirus*, but only viruses of the *Coronavirus* genus have been reported to infect birds. Coronaviruses are enveloped, pleomorphic but usually spherical virus particles of 120–140 nm in diameter. The important antigenic and functional proteins are present as distinct, club-shaped projections (known as spikes), spaced widely apart and dispersed evenly over the surface of the virion. In addition to avian species, coronaviruses have been reported to infect cattle, dogs, cats, pigs, rodents and humans. Coronaviruses are divided into three groups, based on antigenic relationships and gene sequencing data. All known avian coronaviruses are placed in group 3, although there is some experimental evidence to suggest that a group 2 coronavirus, *Bovine coronavirus*, can infect turkeys and cause enteric disease in them. The possibility exists, therefore, that birds may be infected with coronaviruses belonging to other groups.

The best-known avian coronavirus and the most important economically and from a welfare point of view, is avian *Infectious bronchitis virus* (IBV; Fig. 28.1), the type species of the *Coronavirus* genus and a major pathogen in chickens. However, coronaviruses of turkeys, pheasants and other avian species are now recognized.

INFECTIOUS BRONCHITIS

Infectious bronchitis was first reported in North Dakota, USA, in 1931 as an acute, highly infectious respiratory disease of chickens. It now has worldwide distribution. The primary target organ is the respiratory tract, where initial infection and disease occurs. IBV also affects egg-laying performance, and renal damage associated with infectious bronchitis has become increasingly important, particularly in broilers. Economically, the most important aspects are the effects on egg production and quality in laying hens and production performance in broilers, where the initial respiratory infection is frequently exacerbated by secondary infections.

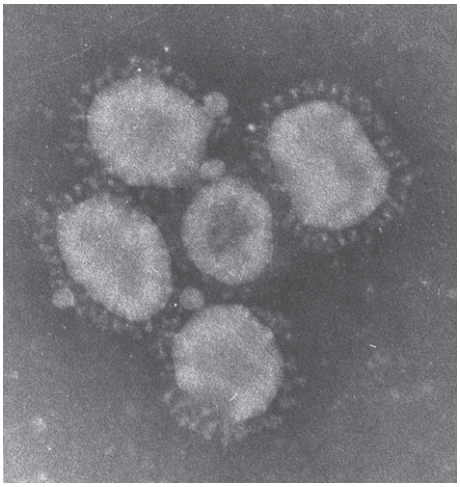

Fig. 28.1 *Infectious bronchitis virus* negatively stained. Electron micrograph courtesy of the Institute for Animal Health, UK.

EPIDEMIOLOGY

Cause

The IBV contains four structural proteins: a surface projection, known as the spike, a nucleoprotein, a membrane protein and a small membrane protein. The spike (S) glycoprotein is responsible for attachment to and fusion of the virus membrane with the host cell membrane, as well as for inducing protective immune responses. Molecular studies have shown that the *S* gene of IBV is also responsible for determining the serotype or genotype of an IB virus or variant. It is now known that only a small number of amino acid changes in the S1 part of the spike can result in what is defined by laboratory tests as a new variant. Since it is possible for concurrent infection with more than one type of IBV to occur, new variants may emerge as a result of both recombination between two different IBVs, as well as by mutations of the genome. Many different IBV variants are recognized both on the basis of antigenic variation, determined by virus neutralization (VN) tests, and increasingly by molecular analysis of the genome (genotyping). Variants of economic importance include: Massachusetts, which is the serotype most commonly found worldwide; Connecticut, Arkansas and Delaware 072 from the USA; T from Australia; D274; D1466; 793B (4/91); B1648; Italian 02 from Europe, and so on. IBVs in Australia have evolved in their own distinct lineages and other areas, for example parts of south-east Asia, have their own unique IBV variants, as well as ones found in other parts of the world. The number of new IBV variants continues to increase as simpler methods for their detection become available. While these different variants are important epidemiologically, their significance in terms of control of infection is less clear. This is because new variants are defined by a very small number of amino acid changes, so that the majority of the genome is conserved.

In addition to antigenic variation within IBVs, variations in virulence are now reported. There have been reports from the USA and Europe of IBV strains with increased virulence compared with previous isolates of the same serotype. There may also be variations in pathogenesis. One such example of this is the 793B (4/91) variant, which has been associated with both muscle

myopathy and scouring in broilers, as well as with mortality in adult breeding hens. More recently a variant IBV isolated in Europe, designated D388 (QX), has been associated with 'false layers'.

Hosts

Although domestic fowl have usually been regarded as the exclusive host of IBV, there are reports of IBV isolations from other avian species. In turkeys, coronaviruses (TCoV, also called *Bluecomb disease virus* and *Turkey enteric coronavirus*) are known to be associated with enteric disease, mortality and underperformance and to affect egg-laying performance in older birds. In pheasants, coronaviruses have been associated with respiratory disease and renal problems. Sequence data for coronaviruses from turkeys and pheasants show them to be at least as closely related to IBV as different IBV strains may be to each other and it is clear that the host range of IBV itself extends beyond the chicken.

In the last few years, possibly as a result of renewed interest in coronaviruses with the emergence of the severe acute respiratory syndrome (SARS) virus, coronaviruses have been reported in other gallinaceous birds, including partridge, guinea fowl and peafowl, as well as in nongallinaceous birds such as teal (in this case, the virus was possibly an IBV that had spread from nearby chickens). In some cases, analysis of the genome of these coronaviruses has shown them to be IBV, and examples exist to indicate both reisolation of a vaccine strain and isolation of a virulent field strain. However, some recent coronavirus isolates from gallinaceous birds, for example mallard duck and a pigeon, have two small extra genes near the 3′ end of the genome, indicating that they cannot be classified as IBV and may represent new species. This is a new area of investigation and, while the coronaviruses detected in some gallinaceous and nongallinaceous birds have not so far been associated with disease, these species are potential carriers of IBV and other coronaviruses and could therefore play a role in global transmission of infection.

Avian coronaviruses are not known to pose any human health risk.

Spread

IBV is highly infectious and only a few virus particles may initiate an infection. After a short incubation period of 1–3 days, bird-to-bird transmission occurs rapidly and signs are seen in most birds in a susceptible flock. Virus is shed via both the respiratory tract and the faeces and a high standard of biosecurity is essential to minimize both entry of infection to a flock and spread between flocks. The virus may be shed for several weeks after clinical recovery and can persist in the intestinal tract for several months. The virus is rapidly killed by common disinfectants but its survival in the environment has not been adequately studied. Direct airborne transmission of virus from the respiratory tract to susceptible birds is probably the most common method of spread, particularly when respiratory signs are present. However, transmission through infected faeces is also important and spread by fomites certainly occurs. The greatest source of infection is birds in which virus is rapidly replicating. While true egg transmission is not believed to be significant, surface contamination of eggs is likely to occur. Because the virus may persist in the bird for many weeks, carrier birds may exist. There is no evidence for the involvement of vectors in IBV transmission.

DIAGNOSIS

Clinical signs

Respiratory signs are the first and most common clinical manifestation in birds of all ages and include tracheal rales, gasping, sneezing, watery nasal discharge, sometimes accompanied by

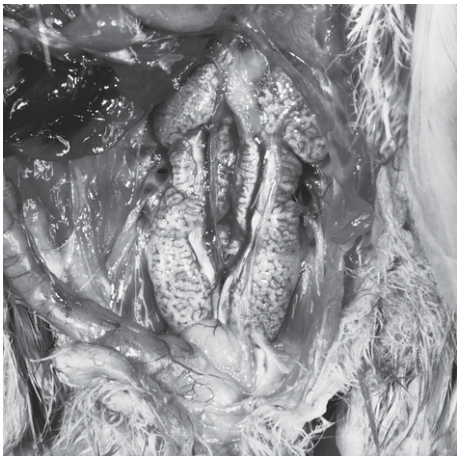

Fig. 28.2 Kidneys of a chicken experimentally infected with an *Infectious bronchitis virus* isolate capable of causing nephritis.

lacrimation, and facial swelling. Generally birds appear huddled and depressed; food conversion and weight gain are usually affected. Mortality is generally negligible in the uncomplicated disease, except in very young chicks and in the absence of maternal antibody. However, significant morbidity and mortality may occur as a result of secondary bacterial infection. The uncomplicated disease may last 10–14 days but secondary infection may increase the duration.

Renal problems caused by IBV often follow an initial respiratory infection in broilers. Affected birds, commonly at 3–6 weeks of age, show depression, scouring and wet litter, associated with increased water intake. In the mild form there may be little or no mortality, but mortality can reach 30%. The kidneys are often pale, swollen and blotchy and the distended tubules are white, because of the presence of urates (Fig. 28.2).

In IBV infection of commercial layers or broiler breeders, respiratory signs may or may not be observed and the most common manifestation is the effect on egg production and egg quality. Flocks infected prior to or during lay are affected. In unprotected flocks, the drop in egg production may exceed 50%. However, in birds that have received a complete vaccination programme but are challenged with field strains against which those vaccines do not provide complete protection, the disease may present as failure to lay at the full potential or production falls of up to approximately 10% (Fig. 28.3). Production may take 4–6 weeks to return to normal, or this may never be achieved.

As production begins to increase, deterioration in external and internal egg quality is seen. Eggs may be smaller than normal; they may be misshapen or show ridging. The shells may be depigmented, some becoming almost completely white (Fig. 28.4), or have calcareous deposits. Eggs are often very thin-shelled or completely shell-less. Internally, the albumen loses its viscosity ('watery whites') and the chalazae are often broken so that the yolk floats free. Small haemorrhages may be seen in the albumen or yolk. Some birds in an infected flock may lay normally, despite showing respiratory signs.

It has been known for many years that infection of very young, susceptible chicks with a particularly virulent IBV strain may be followed by aberrant oviduct development. There may be partial, or almost complete absence of the duct, or vestiges that are nonpatent or cystic. At maturity affected birds may ovulate normally, with the ova then being shed into the body cavity. Such birds go through the motions of oviposition but fail to lay ('blind or false layers'). Sometimes

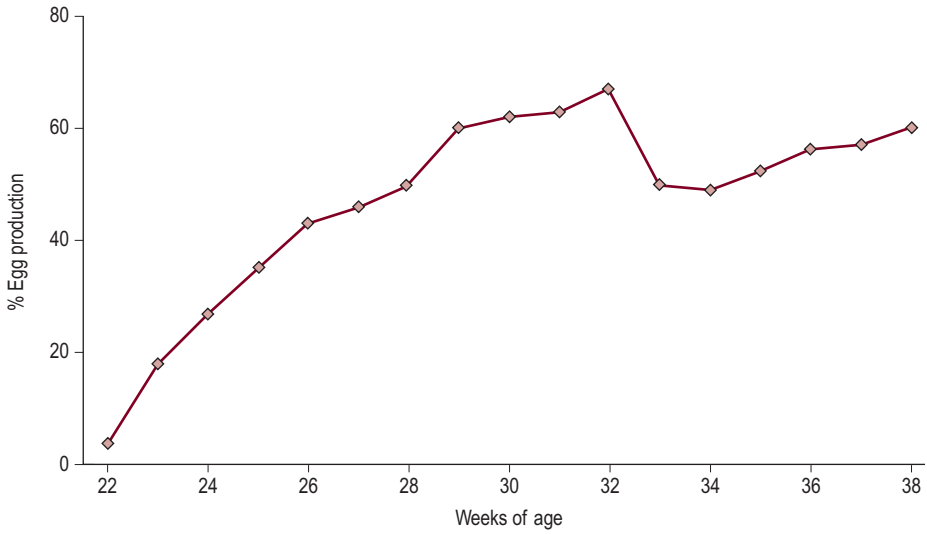

Fig. 28.3 Egg production (%) of a flock of laying hens vaccinated against infectious bronchitis from which a variant *Infectious bronchitis virus* was isolated at 32 weeks of age. (Redrawn with permission of Taylor & Francis from Cook J K A 1984 The classification of new serotypes of infectious bronchitis virus isolated from poultry flocks in Britain between 1981–1983. Avian Pathol 13: 733–741; www.tandf.co.uk/journals)

Fig. 28.4 Eggs laid by different hens (columns) prior to inoculation with a variant *Infectious bronchitis virus* (top row) and between 2 and 7 days later (rows 2 to 5). (Reproduced with permission of Taylor & Francis from Cook J K A, Huggins M B 1986 Newly isolated serotypes of infectious bronchitis virus: their role in disease. Avian Pathol 15: 129–138; www.tandf.co.uk/journals)

ova pass along a patent but abnormal oviduct, giving eggs of reduced shell and albumen quality. Until recently, there was little evidence to suggest that this phenomenon was a significant problem under normal commercial conditions. However, since 2004, there have been reports from parts of Europe of poor egg production in otherwise apparently healthy flocks, but in which some hens may present with a pendulous abdomen. In such cases, ovaries may appear to be normal and

functional, whereas the oviduct is thin-walled and frequently contains large, watery cysts. A novel IBV variant (designated D388 or QX) has been detected in a number of such cases; however, experimental reproduction of the condition with this isolate has yet to be reported.

Lesions

Gross lesions

These include excess mucus in the trachea, nasal cavity and sinuses, accompanied by inflammation and catarrhal exudate that may become caseous. This may lead to formation of mucoid plugs of pus in the primary or secondary bronchi, frequently causing asphyxia. Lungs may be congested and air sac walls may be cloudy and thickened, often with yellow, caseous exudate.

In infected layers, the oviduct may appear normal. However, deposits of yolk may accumulate in the abdomen: so called 'egg peritonitis'.

Histological lesions

Within about 18 h of infection, the trachea and bronchi show loss of cilia with epithelial hyperplasia and metaplasia, often with sloughing of the surface cells. Subepithelial thickening is marked, with oedema and massive monocyte and lymphocyte infiltration of the lamina propria and loss of mucous glands.

Disease of the mature oviduct results in regression in size with metaplasia of the epithelium, glandular dilatation, infiltration of subepithelial tissues with monocytes and proliferation of lymphoid follicles and later fibroplasia. When aberrant oviduct development follows infection with a highly virulent strain at a very young age, there is hypoplasia of the epithelium and tubular glands and the lumen may be obliterated.

In renal infections, interstitial lymphocytic infiltration occurs, with granular degeneration, vacuolation and necrosis of the tubular epithelium, together with accumulation of urates and necrotic material in the lumen. In the ureters there is metaplasia and necrosis of the epithelium with sloughing into the lumen. Visceral gout is sometimes seen.

Host factors

Some innate increase in resistance to infectious bronchitis infection occurs with increasing age. The immune status of the host influences protection, and both maternal immunity and active immunity, resulting from natural infection or vaccination, may prevent or reduce disease and limit virus excretion. Experimental data suggest that there are differences between inbred lines of chicken in their susceptibility to IBV infection, but there are no data to suggest that this extends to commercial breeds. There is evidence that onset of lay (probably associated with hormonal changes) can cause re-excretion of virus that has been latent in the host following an earlier infection.

Factors influencing disease

In young chickens, particularly broilers where stocking density is highest, infection with other pathogens may result in more severe and prolonged respiratory disease. Such pathogens include *Newcastle disease virus*, *Infectious laryngotracheitis virus*, avian pneumoviruses, bacteria such as *Haemophilus paragallinarum* or *Escherichia coli*, and *Mycoplasma gallisepticum* or *Mycoplasma synoviae*. Chilling in brooding chicks can exacerbate the disease, as can poor ventilation and build-up of ammonia levels in the shed. Immunosuppressive agents, such as *Infectious bursal*

disease virus, may reduce the protective immune response to vaccination or field challenge. In the nephritic form of the disease, some breeds appear to be more severely affected and a high-protein diet is an exacerbating factor.

Clinical diagnosis

The clinical features and gross and histological lesions are not diagnostic. The respiratory infection may resemble diseases caused by other pathogens, either alone or as part of a multifactorial disease syndrome. Poor egg production and quality may be caused by many infectious and noninfectious factors, including poor management. There are also other causes of abnormal oviduct development, and kidney disease may be associated with nutritional deficiencies or be of unknown cause. Therefore, proof of IBV infection depends on detecting either the virus itself or an increase in specific antibody levels in serum.

Virus isolation/detection

The detection of IBV in respiratory tract tissues is easiest in the early stages of infection, when virus is replicating most rapidly. Beyond about 7–10 days after infection virus is difficult to isolate. At later stages, or when cases of aberrant egg production are being investigated, faeces, intestinal tract tissue (possibly caecal tonsil) or kidney are the material of choice for virus isolation attempts. Oviduct is not a rewarding site from which to attempt IBV isolation. Sentinel birds have been used successfully to aid the isolation of IBV. Specific-pathogen-free (SPF) chickens, possibly vaccinated against the IBV serotypes common to the area, are introduced into a flock and sampled at weekly intervals thereafter.

For attempted virus isolation from field material, the most successful systems are embryonated chicken eggs inoculated via the allantoic route or chick embryo tracheal organ cultures (TOCs). Material is homogenized with broth containing antibiotics and centrifuged or filtered to remove debris and contaminants. The material is then inoculated into the allantoic cavity of 9–11-day embryonated chicken eggs or into TOCs. Following embryo inoculation, IBV if present, will cause characteristic embryo dwarfing and curling by the 18th day of incubation, but several 'blind' passages, to allow embryo adaptation of the virus, may be required. Three or four passages are therefore usually given before a sample is discarded as negative, making it an expensive and time-consuming procedure. In TOCs, IBV causes ciliostasis within 2–3 days of inoculation. This is easily observed by low-power microscopy. As with embryo inoculation, two or three passages at 1–2-day intervals may be necessary. The identity of the virus isolated in either system should be confirmed. This may be achieved by examining centrifuged allantoic fluid or TOC supernatant by electron microscopy, immunochemical assays, antigen-detecting enzyme-linked immunosorbent assays (ELISAs) or increasingly by using molecular methods. By incorporating serotype-specific monoclonal antibodies into an assay it is possible to identify the IBV serotype involved.

In situations where it is not essential to actually obtain an isolate of the virus, IBV may be detected by immunochemical assays (immunofluorescence or immunoperoxidase using an anti-IBV serum) and increasingly by molecular methods. These involve the reverse-transcription polymerase chain reaction (RT-PCR) together with restriction enzyme fragment length polymorphism (RFLP) analysis, DNA probes or nucleic acid sequencing. They are increasingly used for the detection of IBVs generally, as well as for the differentiation of specific variants. These techniques have also facilitated molecular epidemiological studies to monitor the distribution of specific variants. While direct sequencing of the genome is the most accurate method for identifying new variants, RFLP, which identifies sites unique to a particular serotype, is acceptable

and has provided a rapid method for variant identification. The RNA required for these techniques can be extracted directly from either swab material or appropriate tissues of infected birds, or from allantoic fluid or TOC supernatant of samples from which virus isolation is being attempted. Since viral nucleic acid is what is being detected, it is not necessary for live virus to be present and RT-PCR probably detects the presence of IBV for longer than is possible by virus isolation methods. While, for many purposes, the lack of a live virus is not a problem, for epidemiological studies and for further research, the availability of an isolate could be necessary.

For use in genome analysis, universal oligonucleotide primer sets, designed to detect all known IBVs, as well as ones designed to detect only specific serotypes, have been developed. Initially these were based on sequence data from parts of the spike protein gene. However, as this is the part of the genome showing the most variation, sequence data from parts of the membrane and nucleoprotein genes have also been used. More recently, effort has concentrated on the use of the 3′ end of the genome, an area known to be conserved in coronaviruses. By so doing, it is hoped to develop assays that will detect and differentiate not only IBV strains but also novel coronaviruses of other avian species.

Molecular techniques give results more quickly than virus isolation methods. However, their sensitivity means that great care is needed to avoid contamination of reagents and the tests should be performed only in a dedicated laboratory where a high level of technical expertise is available.

Serology

The methods available to detect IBV antibodies include agar gel precipitation (AGP), haemagglutination inhibition (HI), immunofluorescence, ELISA and VN. The VN test is the most reliable test for identifying and differentiating IBV variants but is time-consuming and expensive and is only performed when it is important to identify the particular serotype involved in an outbreak, or for epidemiological studies. It can be performed in indicator systems such as chicken embryos, TOCs, or avian cell culture using a cell-culture-adapted virus. The HI test is a simple and reliable test but the virus must first be treated with an appropriate enzyme. When performed carefully with appropriate controls, the HI test can differentiate between responses to particular serotypes. However, following exposure to more than one IBV serotype, differentiation by HI becomes unreliable. The other assays all detect group-specific antibodies. The AGP test is simple to perform but relatively insensitive. Some birds never develop precipitins while in others they are present for only a few weeks. For this reason, the only advantage of this test is that a positive result indicates recent exposure to the virus. The ELISA detects all known IBV serotypes and therefore cannot be used to identify particular variants. It is the most commonly used test for monitoring responses to vaccination and to indicate possible field challenge. Because of the availability of reliable commercial kits, it is widely used for flock screening.

CONTROL

Attention to management factors, such as temperature and ventilation, are essential, particularly in controlling the effects of secondary bacterial infection. The use of antimicrobials may also be beneficial against secondary infections. However, drug therapy is of no value in controlling the virus. While strict biosecurity is an important part of disease control, because IBV is ubiquitous

and spreads rapidly it is impossible to exclude it completely by hygienic means. Thus control depends on increasing the resistance of the bird by vaccination.

Live attenuated and inactivated (oil-adjuvanted) vaccines are highly effective and widely used. The live attenuated vaccines are used to prevent and control infection in young birds and to 'prime' future breeders and layers prior to administration of inactivated vaccines. It is important to remember that, for inactivated vaccines to be effective, chickens must have been 'primed' with a live vaccine. To achieve optimal benefit from the inactivated vaccine, at least 4–6 weeks should elapse between the last application of live vaccine and administration of the inactivated vaccine. Ideally the live vaccine should contain IBV serotypes that will stimulate protection against the variants existing in a particular area. The Massachusetts serotype is the one most commonly included but, depending on local circumstances, other serotypes, where this is permitted by the regulatory authorities, may be included in order to optimize protection. Frequently, two or more applications of live attenuated vaccine are given, often varying the serotype of IBV in the later vaccinations.

Despite the apparent protective effect of maternally derived immunity in very young chicks, live vaccines can be administered successfully from 1 day of age by coarse spray, beak dipping or nasal or eye drop. Older birds may be vaccinated via the drinking water, by eye drop or coarse spray. Different vaccination protocols are available, designed for different types of bird (see Ch. 5) but the most important point is to ensure that the vaccine is carefully and correctly administered so that each bird receives the required dose. Whatever protocol is followed, a highly attenuated live virus vaccine is given initially. For broilers this is likely to be given in the hatchery by coarse spray. Revaccination of broilers, possibly at 2–3 weeks of age, is now common practice in some areas. In order that protection against both infectious bronchitis and Newcastle disease may be achieved by one application of vaccine, the two vaccines may be combined. Because of the risk that, if it is present in excess, the infectious bronchitis vaccine may interfere with the response to the Newcastle disease vaccine, the use of a combined product is preferable to the use of two separate vaccines given together. For future breeders or layers, the first vaccination is usually given at about 3 weeks of age, in the drinking water or by spray, and may be followed by one or more further applications of live attenuated vaccine. Before onset of lay an inactivated vaccine is given intramuscularly or subcutaneously. Increasingly, the inactivated vaccine is likely to be a multivalent one containing possibly two different IBV antigens, as well as antigens to other important poultry pathogens.

Excellent protection against homologous challenge is obtained, provided that vaccination is carried out carefully. However, there is now increasing evidence that licensed vaccines provide heterologous protection against challenge with some different IBV serotypes or variants, although it is not currently possible to predict when this might occur based on either serotyping or genotyping. Recent data suggest that, if two antigenically distinct live-attenuated IBV vaccines are applied, preferably separated by at least 2 weeks, good protection may be achieved against challenge with a range of heterologous IBV serotypes. This is probably because of the very small number of amino acid differences between different serotypes or variants in the immunity-inducing part of the virus.

Although a high antibody titre following vaccination or natural infection is indicative of protection, particularly against the homologous strain of IBV, a low titre, even against homologous strains, may not indicate poor protection. This is because local and cell-mediated immunity are very important in protecting against IBV challenge but are difficult to measure by currently available methods. Locally produced antibody in the upper respiratory tract is the important first line of defence against IBV challenges. It is therefore very important that the live attenuated infectious bronchitis vaccines are applied very carefully to ensure good, even stimulation of local antibody in every bird.

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