CEVA HANDBOOK
OF POULTRY DISEASES

8 INFECTIOUS BRONCHITIS

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INFECTIOUS BRONCHITIS

DEFINITION

Infectious Bronchitis (IB) is an acute contagious disease caused by a coronavirus accompanied by respiratory signs in growing birds and disturbance in the quantity and quality of eggs produced by laying hens. Some viral strains are nephropathogenic and induce interstitial nephritis.

HISTORY

In 1931, Schalk & Hawk described a previously unknown respiratory disease in chickens aged 2 to 20 days in North Dakota, characterized by dyspnea and lethargy. Two years later, the causative organism was shown to be a filterable agent (virus). Due to the successful transmission by filterable material and reproduction of respiratory signs, the disease was considered to be a form of infectious laryngotracheitis (LT) (Fabricant, 1998). This confusion was corrected several years later by cross-immunity tests in chickens which demonstrated that the IB virus was different from that causing LT (Beach & Schalm, 1936). The authors also demonstrated that the serum of a bird surviving IB could neutralize the infectiousness of the virus. By the end of 1930s, the economic impact of IB related to egg production losses in laying hens was evident (Delaplane et al., 1939).

The pathology of IB-infected chickens and some epidemiological aspects were described by Hofstad (1945a; 1945b). The spread of the respiratory form of Newcastle disease in the mid 1940s caused serious problems for its differentiation from IB. This spurred the development of better diagnostic methods allowing for isolation and serodiagnosis of the IB virus (Fabricant, 1998). The observations that IB-infected chickens showed only mild respiratory signs before the onset of sexual maturity, and became immune to the infection after the beginning of egg laying, resulted in development of a programme for flock immunisation during the growth stage (Van Roeckel et al., 1942). Another important discovery was the finding that IB etiology included more than one serotype, and that strains were immunologically different (Jungherr et al., 1956).

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

The etiological agent is an RNA virus belonging to the family Coronaviridae. The virion has a pleomorphic shape and a size range of 120 to 160 nm. It consists of an envelope and ribonucleoprotein. On the surface, clavate peplomers give the specific appearance of a solar corona. The genome consists of single-molecule single-stranded linear infectious RNA (Zarkov, 2003). These viruses are poorly resistant in the environment. They can survive for 24 to 72 hours at 37°C and only 15 min at 56°C. At 70°C they may survive for up to 3 months. Organic solvents and common disinfectants (1% formalin, phenol, cresol) inactivate them rapidly.

Viral replication occurs in the cytoplasm for about 5 hours. The consecutive stages of replication include binding, penetration, deproteinisation and transcription, replication and translation. Although the IB virus causes a respiratory disease, some strains can replicate in other non-respiratory cells, for example in kidneys or the genital tract, where they may also induce pathological alterations.

The replication of other strains occurs in the intestinal mucosa, resulting in faecal shedding of the virus without clinical disease (Cavanagh, 2007). The evolution of the virus is related to extensive genetic variations including deletion, insertion, mutation and, occasionally, recombination. These variations are continuous and result in the appearance of multiple phenotypes in relation to pathotypes and immunotypes.

Furthermore, it has been shown that IB viral isolates are a mixture of genetic mutants, generated by genetic mutations and recombinations (Montassier, 2010). Several antigenic variants with different pathogenicity and virulence have been identified: the Massachusetts strain, associated with respiratory pathology, which is used for preparation of vaccines after attenuation; the Connecticut strain, isolated from birds with mild respiratory signs and is used for production of vaccines; the pathogenic «Holland» strain, affecting the respiratory tract and the kidneys; the Arkansas strain, associated with respiratory pathology; the Beaudette strain, a laboratory mutant of the Massachusetts strain which is not

pathogenic for birds; and the T strain, associated with acute nephritis in Australia and possessing a partial antigenic similarity with the Massachusetts strain (Zarkov, 2003).

EPIDEMIOLOGY

The virus is spread on a global scale, and its pathological impact makes it one of most important challenges facing the poultry industry. Diseased chickens are the source of infection, spreading IB by the aerogenous route, as well as some spread via feed and water. Adult birds also represent a major source of infection, as symptomless carriers shedding the virus in oronasal secretions and faeces. A certain stationary pattern is observed, suggesting a continuous excretion of the agent from recovered birds. Re-excretion from hens that have been IB virus-negative for a certain period, after recovering from inoculation at 1 day of age, has also been recorded (Jones & Ambali, 1987). Prolonged and intermittent excretion is a potential hazard for transmission of infection between flocks via personnel or equipment. The existing research on the epidemiology of IB infections shows that the spread of a strain from one region/state to another may result from trade in birds, migrating birds or the use of attenuated vaccines (Cavanagh, 2005). The detection of IBV in non-gallinaceous birds allows us to assume that other bird species could also be vectors of the disease (Cavanagh & Gelb Jr., 2008).

Chickens and hens of all ages are susceptible to infection. The disease is most severe in hatchlings and chickens up to 3-4 weeks of age with clinical respiratory pathology and fatal results. As age advances, the birds become more resistant. The nephropathogenic effect of IBV is usually manifested in chickens older than 4-6 weeks of age, and reproductive tract lesions after egg laying begins (Crinion & Hofstad, 1972; Albassam et al., 1986).

The severity of IB-related respiratory infection is complicated by secondary bacterial or viral pathogens, i.e. *E. coli, Mycoplasma gallisepticum*, Newcastle disease virus etc. resulting in chronic airsacculitis and pneumonia.

CLINICAL SIGNS AND PATHOLOGY

The incubation period of IB is short and dose-dependent; it may be <18 h when the virus is inoculated intratracheally or 36 h after ocular application (Cavanagh & Gelb Jr., 2008).

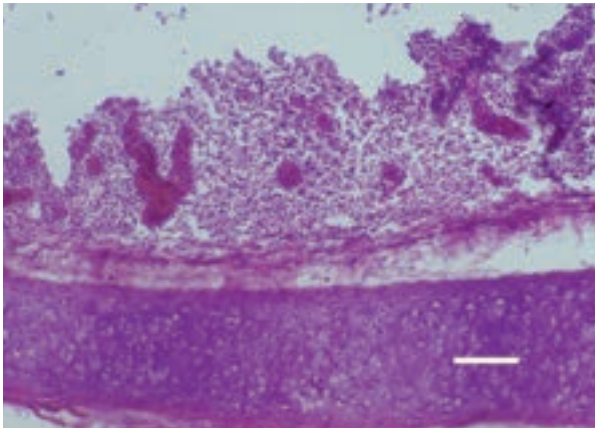

Fig.1

In chickens up to the age of 4 weeks, IB manifests itself in the form of severe respiratory signs (sneezing, coughing, and rales). Rhinites and conjunctivites, depression and crowding around heat sources are observed. The morbidity rate may reach 100%. The mortality in young chickens is usually insignificant unless a secondary infection with a different agent occurs. In such cases, there is a moderate to severe inflammatory cell infiltration of upper respiratory tract mucosa, resulting in thickened and more compact mucosa. In one-day old chickens, IB infection can permanently damage the oviduct, influencing egg production and egg quality during the production period.

Fig.2

In layer hens infected with the IB virus, oophorites and dystrophic necrobiotic lesions affecting primarily the middle and the final third of oviduct's mucous lining are observed. The oviduct is atrophied, cystic, with deposits of yolks or completely formed eggs in the abdominal cavity (the so-called internal layer).

Fig.3

The consequences are drop in egg production, appearance and increase in the number of deformed and pigmentless eggs or eggs with soft shells and watery albumens.

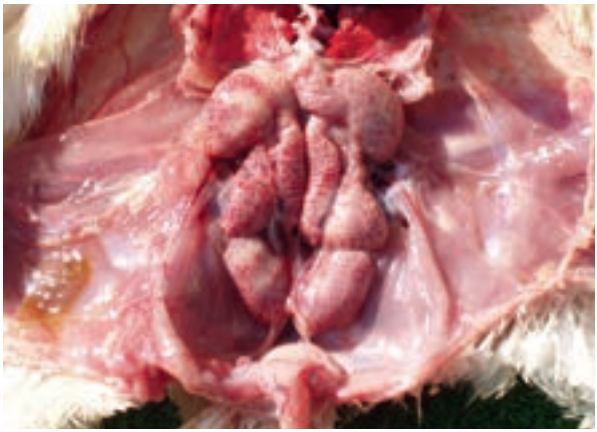

Fig.4

The nephrotropic strains of the IB virus cause severe inflammatory and dystrophic necrobiotic damages of kidneys accompanied by urolithiasis.

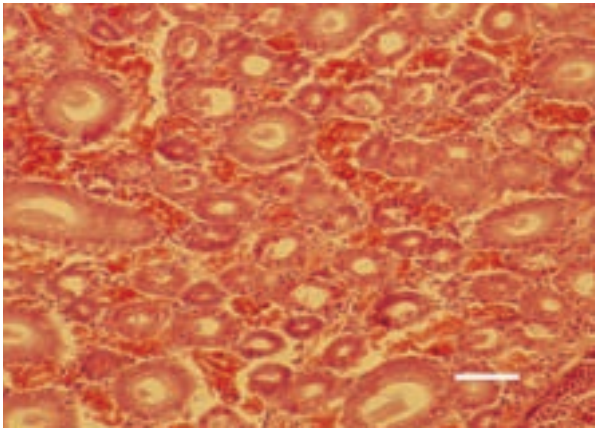

Fig.5

Inflammatory hyperaemia, oedema and haemorrhages in kidneys, due to nephrotropic IB strains. H/E, Bar = 40 µm.

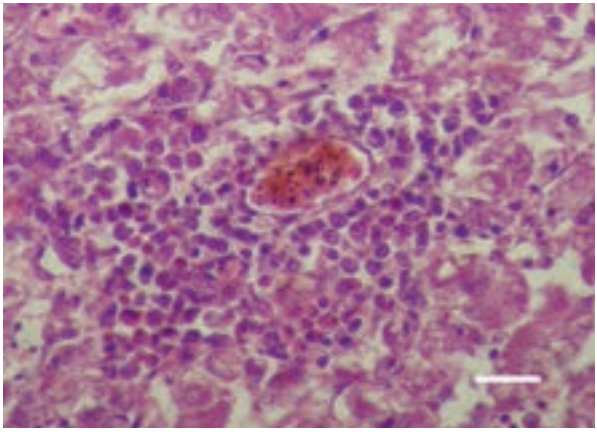

Fig.6

Nephrotropic strains provoke a severe interstitial nephritis. Perivascular focal proliferation of lymphocytes and plasmatic cells in the kidney. H/E, Bar = 25 µm.

DIAGNOSIS

A tentative diagnosis of IB can be made on the basis of history, clinical signs and lesions. This initial diagnosis is confirmed by identification of the agent or via serological tests. If possible the diagnosis should identify the virus' serotype or genotype, in light of the extremely high rate of antigenic variation among IBV strains and the different serotype-specific vaccines (Cavanagh & Gelb Jr., 2008).

Samples for IBV identification should be collected as soon as possible after the appearance of clinical signs, immediately frozen and transported cooled. The cooling regimen should be maintained from the moment of collection right up to delivery to the lab. As noted above, the global spread of the IB virus makes its control exceptionally difficult due to multiple and incessantly emerging serotypes and variants which make cross-protection impossible (Cavanagh, 2007). For live birds, swabs should be taken from the upper respiratory tract. From carcasses, tissue samples from the trachea and the lungs should be used. These samples should then be placed in a transport medium containing antibiotics (penicillin and streptomycin), put on ice and deeply frozen.

In birds exhibiting nephritis or egg production problems, samples should be collected from the kidneys and the oviduct as well. In case of IBV identification by reverse-transcription polymerase chain reaction (RT-PCR), virus isolation is not necessary.

Respiratory or cloacal swabs are suitable for use in RT-PCR tests, not requiring the use of a transport medium. In cases where IB nephritis is suspected, fresh carcass kidney samples should be collected for both histology and virus isolation. Blood samples for serological testing are obtained from acutely ill or convalescent birds.

In order to isolate the virus, after homogenisation and centrifugation of samples (swabs, tissue specimens), the obtained supernatant is inoculated in 9-10-day-old chick embryos, preferably SPF.

Samples are considered positive if abnormalities are present (deformities, dwarfing, haemorrhagic embryos) or embryonic death 5 to 7 days pi. If tracheal organ culture is used, ciliostasis or damage of tracheal

epithelium may be observed 48-72 h p.i. The presence of the virus should however be confirmed by serotyping, genotyping or immuno-histochemical identification (Cavanagh & Gelb Jr., 2008).

Serotyping of IBV via haemagglutination inhibition (HI) and virus neutralization (VN) tests has been conducted in chick embryos and cell cultures (Dawson & Gough, 1971; Alexander et al., 1983; King & Hopking, 1984). Monoclonal antibodies are used in ELISA tests for grouping and identification of IBV strains (Koch et al., 1992).

Genotyping by RT-PCR techniques may largely replace VN and HI tests for detection and identification of field IBV strains. Main advantages of these techniques are the short assay time and potential for detection of various genotypes. The RT-PCR RFLP (restriction fragment length polymorphism) test can be used to identify all possible IBV serotypes as variants of the virus.

The RT-PCR tests in current use provide information about the identity of the virus which is necessary for epidemiology studies in IB outbreaks, however, they may not yield information about the pathogenicity of the strain. Immunohistochemical techniques Employing group-specific monoclonal antibodies may help analysts identify IBV in infected chorioallantoic membranes (Naqi, 1990).

DIFFERENTIAL DIAGNOSIS

Infectious bronchitis should be distinguished from clinical diseases accompanied by respiratory signs and lesions, nephrosonephritis and impaired egg production.

Other respiratory diseases commonly found in poultry include: Newcastle disease, caused by lentogenic respiratory strains, low-pathogenicity avian influenza, infectious laryngotracheitis, swollen head syndrome, *Mycoplasma gallisepticum infection* etc.

Nephrosonephrites caused by nephropathogenic IBV infection are detected in birds older than 4–6 weeks of age. They should be differentiated from infectious bursal disease (Gumboro), mycotoxicoses (ochratoxin A) etc.

The decline in egg production and the bad egg quality in laying

hens should be differentiated from egg drop syndrome 76 (EDS 76).
IB damages the mucosal glands in the middle and last third of the oviduct, whereas EDS 76 – only the last third. This is why IB infection results in impaired quality of egg albumen and eggshell formation, unlike EDS 76 which only affects eggshell quality.

PREVENTION AND CONTROL OF IB

The measures devised to prevent IB outbreaks focus on specific and nonspecific control of the disease. Specific measures include flock vaccination, while nonspecific measures based on the relevant technological norms.

Biosecurity

Management, husbandry and control procedures should comply with the rules commonly accepted for biosecurity in the poultry industry. The sections of the farm allocated to growing birds and birds in production stage must be sufficiently separated, with the different age groups housed separately.
The control of IB in multi-age poultry flocks is very difficult. The production system should strictly apply the "all-in, all-out" principle for depopulation of premises.
The cleaning, washing and disinfection of premises, equipment and transport vehicles should follow a specific biosecurity order, as the removal and management of deep litter.

Vaccination

Both live and inactivated vaccines are used at poultry farms for active immunisation against IB. Live vaccines are generally attenuated. They are used in broiler production for vaccination and revaccination, and in breeder flocks and stock layers for priming vaccination. At present, live vaccines prepared from vaccinal strains of the Massachusetts serotype (H120, B48, Ma5 etc.) are used. Vaccinal strains are usually selected according to the antigenic spectrum of regional isolates (Cavanagh & Gelb Jr., 2008). It is also important to determine the potential for cross protection, mainly applicable for live vaccines. After detection of new serotypes in a region, vaccines of the respective isolates may also be included. In some countries, vaccines based on local isolates are also employed. Apart from Massachusetts strain-based vaccines, those based on the variant 793B strain are applied in some European and Asian countries.

Inactivated oil-adjuvant vaccines against IB are applied as booster vaccines to protect layers and breeder flocks. Inactivated IB vaccines usually include the Massachusetts M41 strain solely or in combination with the so-called "Dutch variants" D274. Most commonly, inactivated IB vaccines are available as polyvalent vaccines that also include ND, IBD and/or other vaccinal strains. After proper vaccination with live and inactivated vaccines, the formed antibodies ensure a prolonged and stable immunity of vaccinated flocks. The efficacy of inactivated vaccines depends on the proper application of priming vaccines

Mass application of live vaccines is generally performed by coarse spray or via drinking water (Andrade et al., 1983). Other routes of administering live vaccines include intranasal or intraocular individual applications, which are time-consuming. Inactivated vaccines require individual application, usually by subcutaneous or intramuscular injection.

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