Technical Update

AVIAN INFECTIOUS BRONCHITIS

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INTRODUCTION

Avian Infectious Bronchitis (IB) is a systemic disease with worldwide distribution, primarily affecting chickens, though it has also been reported in turkeys and pheasants. It is caused by the Infectious Bronchitis Virus (IBV), one of the main agents associated with the Avian Respiratory Complex. IBV infects the respiratory, reproductive, and renal systems, causing high morbidity and variable mortality, particularly with nephropathogenic strains and when co-infections occur. Beyond its health impact, IBV has serious consequences for poultry production due to often irreversible damage to the reproductive tract. Consequently, IB is considered one of the most important diseases in poultry production and is included in the World Organization for Animal Health (WOAH) list of notifiable diseases. Currently, IBV control relies on live attenuated and inactivated vaccines, which reduce clinical signs and help control viral loads on farms. However, vaccination success is limited by the high genetic variability of IBV, which can reduce vaccine effectiveness. Moreover, reversion to virulence and recombination events between vaccine and field strains have been reported.

ETIOLOGY

IBV belongs to the family *Coronaviridae* and is related to coronaviruses of public health importance such as SARS-CoV-1, SARS-CoV-2, and MERS-CoV, as well as to animal coronaviruses like porcine epidemic diarrhea virus. Importantly, IBV is not zoonotic and poses no risk to human health. Taxonomically, IBV is classified within the genus *Gammacoronavirus*, subgenus *Igacovirus*. Its genome consists of a single-stranded, non-segmented RNA prone to mutations and recombination, accounting for the frequent emergence of new variants (1) (Figure 2).

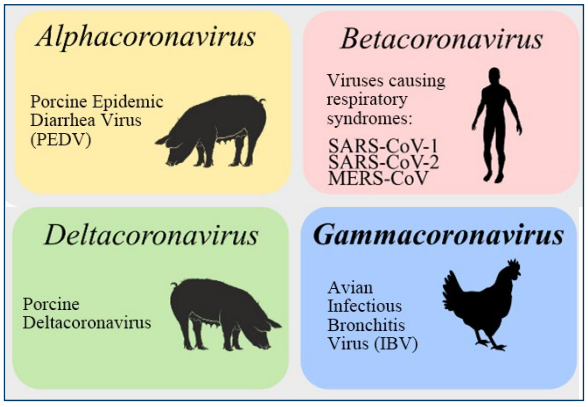

Figure 1. Taxonomic classification of the Avian Infectious Bronchitis Virus and susceptible species.

IBV virions are enveloped, which makes them susceptible to environmental temperature and pH changes, and therefore they are easily inactivated by most detergents and disinfectants. On their surface, they display multiple copies of a protein known as the spike protein (S protein) (Figure 2), which gives the virus its characteristic crown-like appearance.

The genetic characteristics of the S protein—particularly the S1 region—allow for the classification of IBV into genotypes and lineages; thus, the current classification system is based on the phylogenetic relationships of this region (2). Based on this, at least eight genotypes (GI–GVIII), 38 lineages, and multiple intermediate variants—arising from S1 recombination between viruses of different lineages—have been identified (3–6). Of the lineages described to date, 30 correspond to GI viruses (GI-1 to GI-30), while the remaining lineages are distributed among the other genotypes (GII-1, GII-2, GIII-1, GIV-1, GV-1, GVI-1, GVII-1, GVIII-1) (7–9). The phylogenetic organization of the genotypes and lineages identified to date is shown in Figure 2.

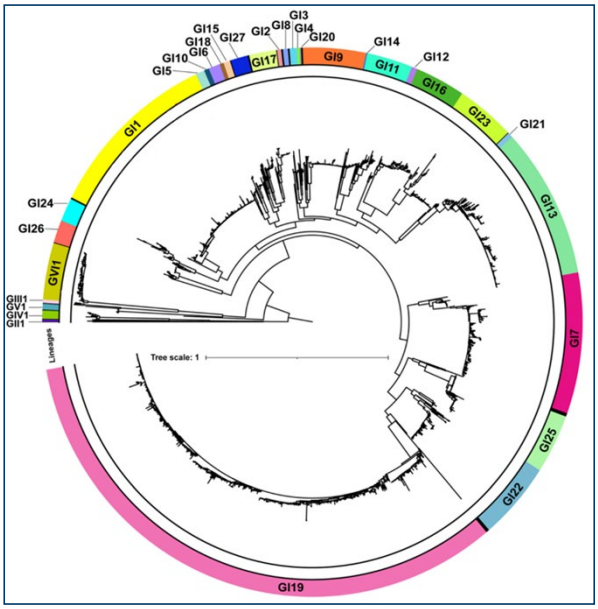

Figure 2. Phylogenetic tree of the genotypes and lineages described for IBV. Adapted from: Ramirez-Nieto et al. (2022).

In parallel, IBV can also be classified according to its antigenic characteristics into serotypes, which are determined by differences in hypervariable regions of the S1 portion of the spike (S) protein (Figure 3), the major antigen of the virus. Although numerous serotypes exist, some of the most well-recognized include Arkansas (Ark), Massachusetts (Mass), Connecticut (Conn), Delaware (Del), Georgia98 (GA98), Georgia08 (GA08), Georgia13 (GA13), 793/B, QX, Q1, among others.

Serotypes are highly relevant because they determine the specificity of the immune response to a particular serotype, although cross-reactivity may occur. Therefore, they must be carefully considered when designing vaccination programs (10) and when interpreting serological test results.

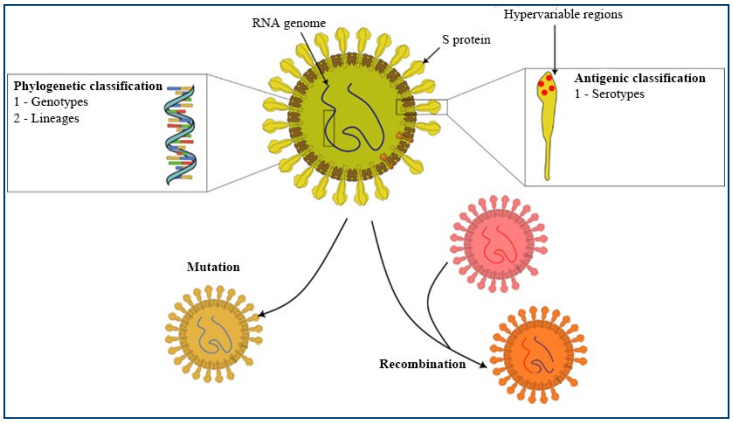

Figure 3. Structure, classification, and main mechanisms of variation and evolution of the Infectious Bronchitis Virus. Illustrations adapted from: ViralZone, SIB Swiss Institute of Bioinformatics.

CLINICAL SIGNS, LESIONS, AND TRANSMISSION

IBV enters primarily via the oro-nasal mucosa. Transmission occurs horizontally via aerosols, respiratory secretions, and fecal matter (figure 4), either by direct contact with infected birds or indirectly through fomites (11).

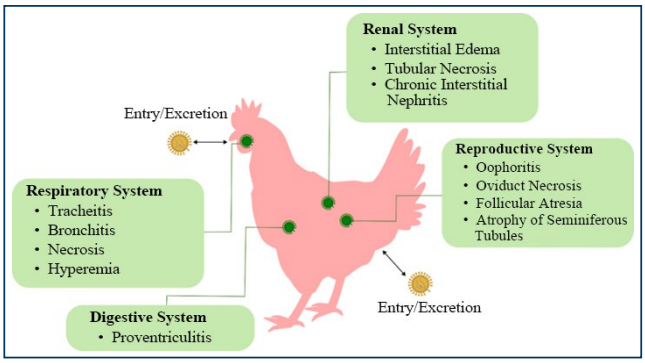

Figure 4. Systems affected and lesions reported in birds infected with IBV.

IBV initially replicates in the epithelium of the upper respiratory mucosa and the Harderian gland, from where it disseminates to the lower respiratory tract. The digestive, reproductive, and renal systems (in the case of nephropathogenic strains) are reached following a viremia that results from the infection of macrophages and monocytes. This systemic dissemination is responsible for the characteristic clinical presentations of Infectious Bronchitis (IB). The severity of clinical signs, as well as the system most affected, depends on the tropism of the infecting IBV strain and the age of the birds. In general, younger birds are considered more susceptible to severe disease.

At the respiratory level, IBV replicates in the oro-nasal cavity, paranasal sinuses, trachea, bronchi, and bronchioles. Viral replication leads to the destruction of ciliated epithelium, predisposing birds to secondary infections with other microorganisms. Affected birds show nonspecific respiratory signs such as sneezing, nasal discharge, conjunctivitis, and swollen head. At necropsy, petechiae and exudate in the airways may be observed, along with pulmonary lesions and airsacculitis characterized by mucopurulent exudate and caseous material (Figure 5). IBV has a short incubation period (18–36 hours), and infected birds usually develop clinical signs 24–48 hours after exposure. Recovery from IBV infection depends on several factors, including the viral strain, the age and immune status of the birds, coinfections, and environmental conditions. IBV infections increase susceptibility to secondary respiratory pathogens or may worsen damage caused by primary respiratory agents (12).

In the reproductive system, IBV replication produces lesions throughout the oviduct, which impair egg production and may cause permanent sequelae. If infection occurs in young pullets, a false layer syndrome may develop, in which oviduct damage prevents egg production. In adult layers, infection causes a transient drop in egg production and alters egg quality, leading to depigmented eggs, misshapen and fragile shells, or albumen abnormalities. If the production drop is mild, normal levels may return within one to two weeks. However, when the decrease is severe, recovery may take six to eight weeks, and in some cases, production never fully returns to normal (12) (Figure 5).

Infections with nephropathogenic strains are characterized by only mild and transient respiratory signs. IBV reaches renal tissue via the bloodstream, producing lesions such as enlargement, edema, diffuse pallor, and urolithiasis. Consequently, affected birds become depressed, show changes in water intake, and mortality spikes may occur.

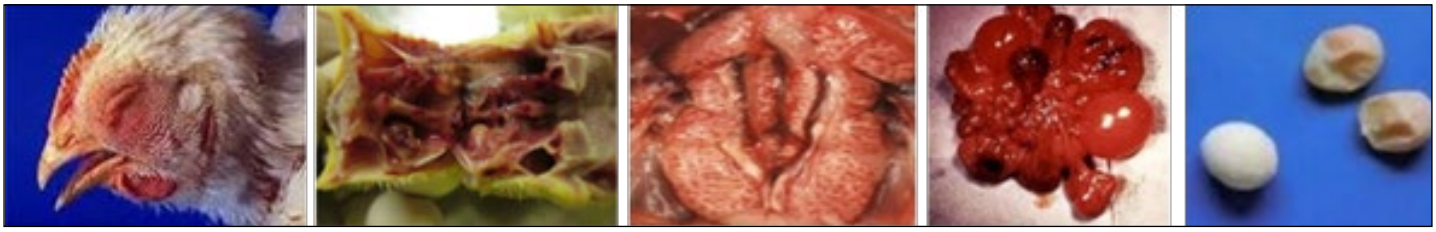

Figure 5. Clinical signs and lesions in birds infected with IBV.

DIAGNOSIS

Clinical signs and lesions caused by IB are not pathognomonic, overlapping with diseases such as Newcastle disease, infectious coryza, laryngotracheitis, mycoplasmosis, avian influenza, and swollen head syndrome (avian metapneumovirus) (13).

The diagnosis of Infectious Bronchitis (IB) can be established directly by detecting the presence of the virus or indirectly through the detection of antibodies using serological tests (Figure 6). For direct detection methods, it is recommended to collect swabs from the upper respiratory tract in live birds, or tracheal and lung tissues in dead birds. If birds show evidence of renal involvement or alterations in egg production and quality, kidney and oviduct samples should be collected in addition to respiratory samples. Viral detection rates are also high in cecal tonsils.

The following section describes the techniques used for both direct and indirect diagnosis of IB. Table 1 provides a summary of these methods along with the recommended sample types.

Virus isolation: Inoculation of suspect samples into 9–11 day SPF embryonated chicken eggs, monitoring for embryo mortality and lesions (hemorrhage, stunting, urate deposits). Alternatively, tracheal organ cultures can be used. Serotyping of isolates is performed using hemagglutination inhibition (HI) or virus neutralization tests.

Molecular methods: In general terms, the amplification target for the detection of specific Infectious Bronchitis virus (IBV) sequences is the gene encoding the S1 glycoprotein, using the RT-PCR technique. However, this result provides no information beyond the presence or absence of the virus and does not allow differentiation of the pathogenicity of the detected viral strain. Therefore, identification and differentiation of viral genotypes (14,15) is necessary, which can be achieved through techniques such as RFLP (restriction fragment length polymorphism) or by sequencing partial fragments or the complete genome. Nucleic acid sequencing combined with phylogenetic analysis provides critical information to determine whether the detected strains correspond to vaccine strains or field isolates.

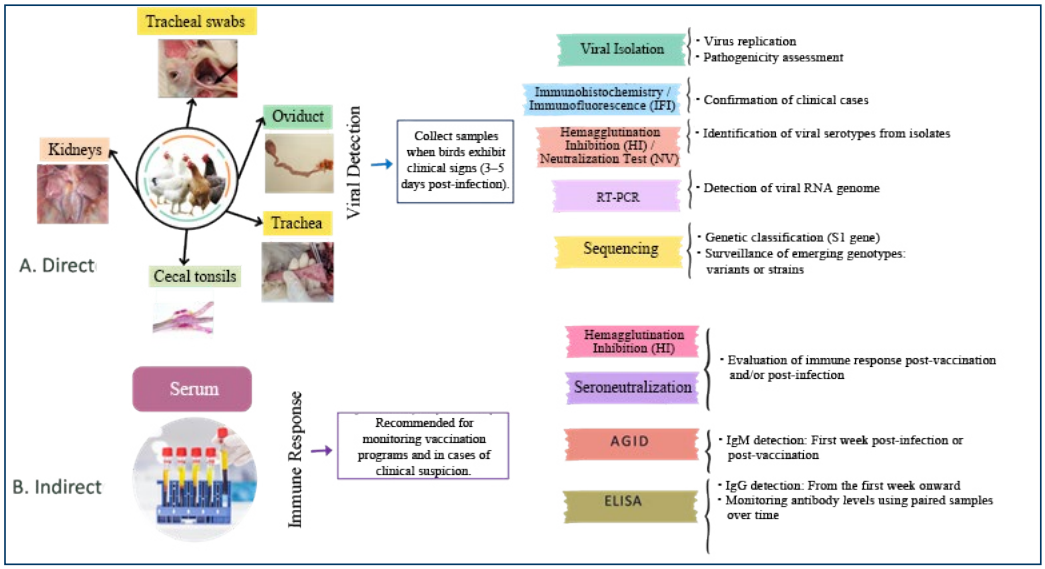

Figure 6. Tests used and their purpose in the diagnosis of Infectious Bronchitis (IB). A. Tests for direct detection. B. Tests for indirect detection.

Serological methods: The techniques used to establish the presence of antibodies against Infectious Bronchitis Virus (IBV) include virus neutralization (VN), hemagglutination inhibition (HI), agar gel immunodiffusion (AGID), and ELISA. The latter is widely used in routine diagnostics due to its ease of application and the availability of commercial kits. One limitation, however, is that although various parameters have been proposed as guidelines for result interpretation, there is no consensus, since specific characteristics of the birds and the production system itself must be taken into account, as these factors can interfere with both the results and their interpretation.

Test Type	Technique	Purpose	Notes
Histological	Immunofluorescence	Detection of viral antigens and serotype identification.	Monoclonal antibodies (MAb) should be used to avoid nonspecific reactions.
	Immunohistochemistry		
Viroogical	Virus isolation	Detection and replication of the virus.	Slow, costly, and labor-intensive process; requires confirmation with other techniques.
Molecular	Endpoint or real-time RT-PCR	Viral detection.	Detects specific viral genes but cannot differentiate between vaccine and field strains.
	Sequencing	Virus characterization.	Enables detection of specific viral genes and genetic differentiation.
Serological	Enzyme-Linked Immunosorbent Assay (ELISA)	Monitoring antibody response post-vaccination or exposure.	Must verify potential cross-reactivity between IBV serotypes.
	Agar Gel Immunodiffusion (AGID)*	Antibody detection.	Depending on the method used, consider cost and complexity of the process.
	Serum Neutralization		
	Hemagglutination Inhibition (HI)*		May lack specificity to distinguish between variants.

**Used for serotyping from viral isolates.*

Table 1. Available tests and recommended samples for the diagnosis of IBV in commercial poultry.

VARIABILITY AND CIRCULATION OF IBV

Infectious Bronchitis Virus (IBV) was first described in North America in 1931 (16), and since then, multiple reports have demonstrated its wide distribution and genetic variability. This variability may have been partly influenced by the introduction of live vaccines, which have impacted the genetic landscape of IBV. Lineages GI-1 and GI-9, where the Massachusetts-type (Mass-type) and Arkansas-type (Ark-type) vaccines are classified, are commonly identified. Additionally, the circulation of vaccine-derived viruses has contributed to recombination events with field strains and reversion to virulence, leading to the emergence of new viral variants of significance for the poultry industry (17).

With respect to geographic distribution, in North America the predominant lineage is GI-17 (DMV/1639/11-type), which appears to have displaced GI-9 and currently co-circulates with GI-27 (GA08-type), both widely used for IB vaccination in that region (18). In South America, the dominant lineage is GI-11, which is region-specific, along with GI-16 (19). However, GI-1 and the Asian lineage GVI-1 have also been reported (6). In Europe, the most relevant genotypes are GI-1, GI-19, GI-13, GI-12, and GII-1 (20,21), while in Asia the predominant lineages have been GI-19 and, more recently, GI-23 and GVI-1 (22–24). In Africa, a region-specific lineage (GI-26) circulates together with others such as GI-14, GI-16, and GI-19, among others (20,25).

More recently, the global IBV landscape has been influenced by the dissemination of GI-23, originating in the Middle East, which spread to African countries in 1998, to Europe in 2015, to the Americas in 2021, and to other parts of Asia (26,27). This lineage appears to be displacing regionally established strains. Initially described in the late 1990s as the "Israel variant 2," it was not associated with high morbidity or mortality. However, the accumulation of mutations and its spread via wild birds have resulted in the emergence of highly nephropathogenic strains (27). These new strains, being antigenically distinct from those circulating in different countries, have spread rapidly, significantly impacting the poultry industry.

Consequently, several countries have needed to review and update their vaccination programs to provide coverage against GI-23. It has been shown that a combination of Mass-type vaccines with serotypes 793B-type and SCZY3-type (QX-like) provides protection against emerging GI-23 strains (28).

Due to the geographic isolation of Oceania, IBV in this region has evolved into lineages and genotypes independently, resulting in the emergence of unique genetic groups. Despite the diversity present in this continent, the main lineages are GI-5 and GI-6 (2,20).

PREVENTION AND CONTROL

As with most infectious agents, the prevention and control of Infectious Bronchitis Virus (IBV) rely on biosecurity programs and vaccination. Regarding biosecurity, these programs should focus on preventing the entry and spread of the virus through flock separation on farms, thorough cleaning and disinfection of facilities and fomites that may serve as vehicles for viral transmission, and the implementation of an appropriate immunization plan for susceptible birds.

The use of vaccines reduces the severity of clinical signs and decreases both the duration and level of viral shedding in infected birds (12). Currently, attenuated live and inactivated vaccines are available. Live vaccines are used in short-cycle birds and for the primovaccination of long-cycle birds, while inactivated vaccines are applied in layers and breeders. Many available vaccines have been developed from Massachusetts-type (Mass-type) strains. However, vaccine selection should be based on the genetic and antigenic characteristics of circulating field strains, as little cross-protection exists among heterologous serotypes.

The lack of cross-reactivity between the multiple IBV variants has complicated the establishment of standardized global vaccination programs. Nevertheless, several studies have demonstrated that vaccination protocols using strains of different serotypes can induce cross-protection against heterologous viruses, creating so-called “protectotypes” (29,30). Currently, the most widely used protocols combine Mass-type strains (Ma5 or H120) with 793B-type strains (4/91 or CR88), which confer protection against a broad spectrum of viruses. Although the underlying principle of protectotypes is not fully understood, it is hypothesized that stimulating the immune system with different antigens may have an additive effect, enhancing both innate and adaptive antiviral responses (31). For this reason, protectotype protection can only be achieved through the use of live attenuated vaccines. However, there is evidence suggesting that the use of inactivated vaccines as boosters may enhance the initial protectotype response (32). Studies indicate that the effectiveness of this strategy is due to increased activation of cytotoxic lymphocytes and the production of broad-spectrum mucosal immunoglobulins, limiting the establishment of field virus infections (33). Despite the advantages of protectotypes in IBV immunization, viral variability must be considered, requiring evaluation of the immune response effectiveness of protectotypes against region-specific strains.

In addition to the challenges posed by vaccines against new IBV variants, their safety has been debated, as evidence suggests that attenuated vaccine strains may revert to virulent forms or favor recombination events (17,34,35). For this reason, new, safer biological alternatives capable of inducing strong protection are being developed, including DNA vaccines and vectored vaccines (36–38).

There is no specific treatment for the disease, but certain measures—such as improving air quality through proper ventilation, avoiding exposure to low temperatures, and maintaining feed intake—help reduce losses in affected flocks. In cases of nephritis, lowering dietary protein levels and supplementing drinking water with electrolytes are recommended, as these measures may reduce mortality. Likewise, in the case of secondary bacterial infections, antibiotic therapy may be required (12).

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