

Avian Infectious Bronchitis

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Abstract: Infectious bronchitis (IB) is one of the major economically important poultry diseases distributed worldwide. It is caused by infectious bronchitis virus (IBV) and affects both galliform and nongalliform birds. Its economic impact includes decreased egg production and poor egg quality in layers, stunted growth, poor carcass weight, and mortality in broiler chickens. Although primarily affecting the respiratory tract, IBV demonstrates a wide range of tissues tropism, including the renal and reproductive systems. This review discusses aspects on the epidemiology of the prevalent IBV strains in a particular region is therefore important to guide control and preventions. Meanwhile previous diagnostic methods such as serology and virus isolations are less sensitive and time consuming, respectively; current methods, such as reverse transcription polymerase chain reaction (RT-PCR), Restriction Fragment Length Polymorphism (RFLP), and sequencing, offer highly sensitive, rapid, and accurate diagnostic results.

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1. Introduction

Avian infectious bronchitis (IB) is an acute, highly contagious viral disease of poultry, clinically manifested by respiratory sign like tracheal rales, sneezing and coughing (Cavanagh and Naqi, 2003). Poultry of all ages can be infected by infectious bronchitis virus (IBV) (Barua *et al.*, 2006). Globally IB is considered as one of the top economically important poultry disease as it reduces egg production, degrade egg shell quality and renders less hatchability and poor body weight gain, and poor feed conversion ratio (FCR) in broiler (Cavanagh and Gelb, 2008). The disease can often be a component of mixed infection along with other pathogen like Mycoplasma and *E. coli* that produces air sacculitis, which may result in meat condemnation during processing (Ali *et al.*, 2015). The IBV under genus *Gammacoronavirus* and family of *Coronaviridae* (Abdel-Moneim *et al.*, 2006). Epithelial cells of respiratory tract, reproductive and urinary tract is the primary target of IBV (Balasubramenia *et al.*, 2013). Some IBV strains can replicate in gastrointestinal tract that can cause up to 44% mortality (Cavanagh, 2005). In some other cases IBV infection in proventriculus may result in 75% to 100% mortality in young birds (Yu *et al.*, 2001). There are more than 20 known serotype within IBV (Mass, Conn, Ark, Geofgia, Delaware-e/072/92 and California) recognized globally (Lee and Jckwood, 2000). On the basis of cross reaction IBV serotypes are different. Most of the serotypes and genotypes of IBV do not produce cross protection (Jackwood, 2012; Cavanagh *et al.*, 1992). In spite of

routinely vaccination against IBV, there may be still disease outbreak in vaccinated flock, as it does not give cross protection against antigenically different serotype and variant strain of virus (Callison *et al.*, 2006). The disease is transmitted through the air, hence it is an air-borne infection, direct bird to bird contact and indirectly through mechanical spread have also been indicated to be a route of transmission (Cavanagh and Gelb, 2008).

2. Etiology and Molecular Biology

Infectious bronchitis is caused by infectious bronchitis virus (IBV), a single stranded positive sense, enveloped RNA virus of 27–32 kb length (Lai and Cavanagh, 1997). The virus has been classified under the *Gammacoronavirus* genus in the family *Coronaviridae*, order *Nidovirales*. Like other members of corona virus family, the IBV genome is composed of structural and nonstructural proteins. Structural proteins include the spike [S] glycoprotein, envelope [E], matrix [M], and nucleocapsid [N]. These proteins together play different roles in viral attachment, replication, and inducing clinical disease. Of major structural proteins, the M protein is the most abundant trans membrane protein, which play vital role in corona virus assembly through interaction with viral ribonucleocapsid and spike glycoprotein (Bande *et al.*, 2015). IBV E protein is, however, scant and contains highly hydrophobic transmembrane N terminal and cytoplasmic C-terminal domains. Studies have shown that the E protein is localized to the Golgi complex in IBV infected cells and is integrally

associated with viral envelope formation, assembly, budding, ion channel activity, and apoptosis (**Wilson et al., 2006**).

3-Pathogenesis

Infectious bronchitis virus infects primarily the respiratory system. However, some variants and several field isolates affect the reproductive, renal, and digestive systems of chickens. Disease pathogenesis differs according to the system involved, as well as the strain of the virus (**Cavanagh, 2007**).

3.1. Host Susceptibility.

Although domestic fowl (*Gallus gallus*) and pheasant (*Phasianus* spp.) are considered to be natural hosts for IBV (**Cavanagh et al., 2002**), other IBV-like corona viruses have been identified in nondomestic avian species including pheasant, peafowl, turkey, teal, geese, pigeon, penguins quail, duck, and Amazon parrot (**Circella et al., 2007**). Antigenic similarities between turkey corona virus (TCoV) and avian infectious bronchitis virus (AIBV) have also been demonstrated (**Guy, 2000**).

3.2. Age and Breed Predisposition.

Chickens of all ages and breed types are susceptible to IBV infection, but the extent and severity of the disease is pronounced in young chicks, compared to adults. Similarly, resistance to infection was suggested to increase with increasing age (**Crinion and Hofstad, 1972**). Experimental evidence suggests that line C white leghorn chickens are more resistant to M41 IBV challenge, compared to line 151, although both lines had similar virus shedding rate (**Bacon et al., 2004**).

3.3. Infection and Transmission.

The virus is transmitted via the respiratory secretions, as well as faecal droplets from infected poultry. Contaminated objects and utensils may aid transmission and spread of the virus from one flock to another. Evidence of virus was shown in trachea, kidney, and Bursa of Fabricius 24 hrs following aerosol transmission (**Cavanagh and Gelb, 2008**). The nature of IBV persistence remains to be elucidated; however, detection of the virus in the caecal tonsils (up to 14 weeks) and from faeces (20 weeks) after infection might suggest a role of faecal shedding in viral transmission and persistence (**Alexander and Gough, 1977**).

3.4. Incubation Period. Generally the short incubation period for IBV varies with infective dose and route of infection. For example, while infection via the tracheal route may take a course as short as 18 hours, ocular inoculation leads to an incubation period of 36 hours (**Cavanagh and Gelb, 2008**).

3.5. Clinical Course and Manifestations.

In the host, initial infection occurs at epithelia of Harderian gland, trachea, lungs, and air sacs. The virus

then moves to the kidney and urogenital tract, to establish systemic infection (**Arshad et al., 2002**). In this regard, the severity and clinical features of IB depend on the organ or system involved. Infection of the respiratory system may result in clinical signs such as gasping, sneezing, tracheal rales, listlessness, and nasal discharges. Affected birds appeared listless and dull with ruffled feathers. Other signs may include weight loss and huddling of birds together under a common heat source (**Cavanagh and Gelb, 2008**). Other clinical outcomes associated with IB infection include frothy conjunctivitis, profuse lacrimation, oedema, and cellulitis of periorbital tissues. Infected birds may also appear lethargic, with evidence of dyspnoea and reluctance to move (**Terregino et al., 2008**). Nephropathogenic IBV strains are most described in broiler-type chickens. Clinical signs include depression, wet droppings, and excessive water intake. Infection of reproductive tract is associated with lesions of the oviduct, leading to decreased egg production and quality. Eggs may appear misshapen, rough-shelled, or soft with watery egg yolk. Unless effective measures are instituted, decline in egg production does not return to normal laying, thus contributing to high economic loss (**Cavanagh, 2007**).

3.6. Gross and Histopathology.

Pathological changes observed grossly at necropsy include congestion and oedema of histopathological changes include loss of cilia, oedema, rounding and sloughing of epithelial cells, and infiltration by lymphocytes. Presence of Russell bodies in Harderian cells has been observed following infection with H120 IBV serotype (**Toro et al., 1996**). Nephropathogenic IBV strains cause nephritis characterized by swelling and congestion of the kidney, sometimes with pallor of ureters that contain urate deposits. Coinfection with bacterial pathogens such as *E. coli* may lead to a more complex outcome, usually associated with high morbidity and mortality. Similarly, infection with nephropathogenic IBV strains may result in pale, swollen, and mottled kidneys (**Cong et al., 2013**). Histological findings include interstitial nephritis, tubular degeneration, and infiltration by heterophils. In some cases, necrotic and dilated tubules are filled with urates and casts (**Cavanagh and Gelb, 2008**). Experimental studies have shown that IBV-T-strain causes necrosis of the proximal convoluted tubule and distension of distal convoluted tubule. In addition, necrotic foci, heterophils, and lymphocytes are observed in the interstitial spaces. Oedema of Bowman's capsule and granulocytic infiltration has been reported in the collecting ducts and spheroids (**Chousalkar et al., 2007**). When the reproductive system is affected, there may be nonpatent and hypoglandular oviduct,

especially in severely affected chickens (**Chousalkar et al.,2007**). Large accumulation of yolk fluid may be seen in the abdominal cavity often associated with bacterial infection in laying hens (**de Wit et al.,2011**).

4. Diagnosis

4.1. Serology.

In the past, serological assays such as virus neutralization (VN) and haemagglutination inhibition (HI) were used widely for detecting and serotyping IBV strains. These tests also have been used to measure flock protection following vaccination (**King and Cavanagh,1991**). Serotype-specific antibodies usually are detected using HI, even though the HI test is less reliable (**OIE, 2008**). On the other hand, ELISA assays are more sensitive and easily applied for field use and in monitoring antibody response following vaccination or exposure. However, emergence of different IBV serotypes that do not cross-react with commonly available antisera generally made serological tests less applicable and nonconclusive in classifying new or emerging IBV isolates (**Kant et al., 1992**).

4.2. Virus Isolation and Identification.

Virus isolation has been the gold standard for the diagnosis of IBV (**Stephensen et al., 1999**). Taking samples during early onset of the disease and ensuring the right sampling techniques are important keys for successful isolation of IBV. To support successful virus isolation from swabs, recommended to place swab sample in buffered solutions of media or PBS before transporting them to the laboratory. If tissue samples are to be collected, recommended tissues are trachea, kidney, proventriculus, tonsil, and oviduct. Tissue samples must be collected aseptically from scarified chickens or immediately upon death, placed in sterile, tightly sealed plastic specimen bags, and transported to the laboratory on ice for further processing (**Gelb et al., 1998**).

different laboratories use various isolation methods, as described below.

4.2.1. Embryonated Chicken Egg.

Most IBV strains grow well when inoculated into the allantoic cavity of a 9–11-day-old chicken embryo. Clinical samples from tracheal swab, broth, or tissue homogenate (10%w/v) are inoculated into the allantoic cavity of specific pathogen-free eggs and incubated at 34–37°C, after inoculation. Eggs are candled daily to monitor embryo viability (**Beaudette and Hudson,1937**). After 5–7 days, inoculated eggs are opened and observed for characteristic IB lesions such as curling and dwarfism of the infected embryo. It is important to note that such findings are suggestive, but not pathognomonic (**Loomis et al., 1950**).

4.2.2. Cell Cultures.

Isolation of IBV has been attempted in various primary and secondary cells, such as chicken embryo kidney fibroblast and Vero cells, respectively. Infected cultures are characterized by rounding, development of syncytia, and subsequent detachment from the surface of the plate (**Arshad,1993**).

4.2.3. Organ Cultures.

Tracheal organ culture (TOC) can be used to propagate both embryo-adapted and non-embryo adapted IBV strains. TOC is prepared from tracheal rings of 20-day-old chicken embryo. The tracheal rings are maintained in a roller bottle and infected with IBV-suspected samples. The culture is observed microscopically for evidence of ciliostasis under light microscope. Complete impairment of ciliary activity usually is considered as a positive culture (**Jones and Hennion, 2008**).

4.3. Electron Microscopy.

Electron microscopy provides a direct means of detecting and identifying IBV in biological samples based on morphological characteristics of corona virus. Positive cultures are confirmed based on the presence of corona virus-like pleomorphic structures with spike projections, following negative staining with phosphotungstic acid. Importantly, the shape and diameter (120 nm) of the virus are taken into consideration when making diagnostic judgments. Apart from the negative staining method, transmission electron microscopy (TEM) is also a useful tool which enables the visualization of virus-like particles in infected cells (**Arshad,1993**).

4.4. Immunohistochemistry.

Immunoperoxidase and immunofluorescence are two important histochemistry methods for detection and confirmation of IBV antigen from infected tissue and/or cells. These methods work based on antigen antibody reactions (**Bezuidenhout et al., 2011**). Immunoperoxidase methods such as the avidin-biotin complex (ABC) have been used successfully to localize IBV antigen in tissue samples. Likewise, indirect immunofluorescent assay is the most frequently used fluorescent technique (**Abdel-Moneim et al., 2009**).

4.5. Molecular Diagnostic Assays.

In view of their increased sensitivity and reduced reporting time, molecular methods, such as Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), real-time PCR, Restriction Fragment Length Polymorphism (RFLP), and genome sequencing, have nearly replaced conventional serology and virus cultivation methods of IBV diagnosis (**Adzhar et al., 1997**).

4.5.1. RT-PCR Methods.

This approach uses viral RNA, amplified either directly (one-step RT-PCR) or following cDNA synthesis (two-step RT-PCR). An RT-PCR assay was

designed and introduced in 1991 for detecting the IBV-S2 gene (Lin et al.,1991). Subsequently, general and serotype-specific RT-PCR assays were designed to target different regions and/or fragments in the IBV viral genome (Keeler et al.,1998). The UTR and N-gene-based RT-PCR are used for universal detection, because of the conserved nature of the target region in many IBV serotypes (Adzhar et al., 1996). A pan-corona virus primer, targeting a conserved region of different corona virus isolates, could also be used in one-step RT-PCR amplification of IBV strains (Stephensen et al.,1999). However, amplification and sequencing of the S1 gene provide a reliable means for genotypic classification of new IBV strains (Zhu et al., 2007). A serotype-specific PCR assay has been designed to enable differentiation of Massachusetts, Connecticut, Arkansas, and Delaware field isolates (Keeler et al., 1998).

4.5.2. Restriction Fragment Length Polymorphism (RFLP).

This is an IBV genotyping method carried out to differentiate different known strains of IBV and to identify new variants following RT-PCR amplification (Lin et al.,1991). Full-length sequence of IBV S1 glycoprotein could be targeted for amplification and enzymes analysis (Mardani et al.,2006). RFLP allows differentiation of various known IBV strains, based on their unique electrophoresis banding patterns defined by restriction enzyme digestion (Montassier et al., 2008). The assay was found to be comparable with traditional virus neutralization assay, although some strains such as the Gray and JMK strains were reportedly difficult to differentiate using arrays of restriction enzymes, thus limiting the universal application of this method (Kwon et al.,1993).

4.5.3. Real-Time RT-PCR and Other Forms of PCR Assays.

For increased test sensitivity and specificity, real-time RT-PCR assays (Acevedo et al., 2013) have been introduced for detecting IBV. Apart from detection, it is possible to quantify IBV viral load from tissue and/or clinical samples by real-time RT-PCR assays based on viral copy number or fold changes (Callison et al., 2006). Likewise, differentiation of Massachusetts from non-Massachusetts is possible by real-time RT-PCR assay targeting S1 glycoprotein gene (Jones et al., 2011). Recently, a high resolution melt curve analysis (HRM) was also developed to allow differentiation of field from vaccine IBV strains as well as for rapid and sensitive detection of recombinant variants (Hewson et al., 2010). Meir et al., (2010) reported that real-time RT-PCR was comparable to virus isolation and one or two times more sensitive in detecting M41 IBV than ordinary N-gene and S1 gene specific RT-PCR assays. On the other hand, real-time RT-PCR was ten fold more

sensitive compared to virus isolation and 30- or 40-fold compared to N-gene or S1 gene-based RT-PCR, respectively. The authors, however, reported variations in sensitivity when either N-gene or S1 genes were targeted as well as when different samples are used for viral amplification. Other forms of PCR methods used in detecting IBV include nested PCR (Adzhar et al., 1996), multiplex PCR (Chen et al., 2010); and reverse transcription loop mediated isothermal amplification (RT-LAMP) (Chen et al., 2010). While these methods are more sensitive than standard RT-PCR, they are more expensive as well and might be beyond the financial capacity of many producers.

5. Differential Diagnosis

Several respiratory diseases, such as Newcastle disease (ND), infectious laryngotracheitis, infectious coryza, avian metapneumo virus (aMPV), and avian influenza (AI), may produce clinical signs similar to avian infectious bronchitis. However, certain clinical features, including neurological signs and diarrhoea in ND, high mortality in AI, and pronounced heads swelling in coryza, are uncommon in IBV infection and thus may be used in ruling out or arriving at narrowed tentative differential list (Cavanagh and Gelb.,2008).

6. Conclusion:

Infectious bronchitis still threat for poultry production due to the appearance of new IB serotypes. The appearance of antigenic variants of infectious bronchitis virus cause a major problem in the poultry industry. So, accurate diagnosis must be performed to determine new serotypes especially during outbreaks and help in control strategies to perform protection studies with these new isolates and help in determining the optimum vaccination.

7-Declarations:

Ethics approval and consent to participate

Yes, (it is a review, not experiment).

Consent for publication

Not applicable.

Availability of data and material

All data collected in this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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List Of Abbreviation**Mean****Infectious bronchitis****Infectious bronchitis virus****Reverse transcription polymerase chain reaction****Restriction Fragment Length Polymorphism****Feed conversion ratio****Avian infectious bronchitis virus****Turkey corona virus****Tracheal organ culture****Transmission electron microscopy****Deoxyribonucleic acid****Abbreviation****IB****IBV****RT-PCR****RFLP****FCR****AIBV****TCoV****TOC****TEM****DNA****References:**

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