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Infectious Bronchitis: A Moving Target for Commercial Poultry Industry

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Abstract

The poultry industry is one of the most efficient and flourishing sectors of agriculture that not only provides cheaper protein (eggs, meat) but also contributes heavily to the country's economy. There are several challenges faced by the poultry industry worldwide. Among all these challenges, disease management has been a major problem. Infectious bronchitis viruses (IBVs) are RNA-based viruses having high recombination and mutation rates. IBVs are gamma coronaviruses affecting the upper respiratory tract of chickens. Due to the high rate of mutation and recombination, IBVs are very difficult to properly diagnose and control. Some serotype IBVs are extremely resistant, causing high economic losses in the form of excessive use of antibiotics after the eruption of secondary pathogens and mortality, but some serotype IBVs are limited to morbidity losses only. There are some control methods for IBVs and practicing effective vaccination and biosecurity measures is highly recommended. Exposure of IBVs to chicken flock postulates gateway to secondary pathogens, which also pass on to coming generations. This review paper provides updated research tools and methods to diagnose and control IBVs.



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Introduction

Infectious bronchitis was first recognized 91 years ago in 1930's in the United States. Today, it's still challenging for the poultry industry around the globe, and negatively affecting economics and the food supply chain resulting in high prices. Initially, symptomatic illustrations described its mild form of the infection called laryngotracheitis (ILT) but in 1936 Beach and Schalm [1] confirmed that the virus strain that caused infectious bronchitis was different from ILT in chicks. The first infectious bronchitis virus (IBV) vaccine was developed in 1937 in the United States [2]. In 1990, the use of molecular techniques revolutionized IBV diagnosis [3]. Its variants are distributed (serotypes and genotypes) in all continents except Antarctica [4, 5]. IBV was first documented by Schalk and Hawn in 1931[6].

Avian gamma coronavirus (Gamma-CoV) is the causal organism of infectious bronchitis virus, which is round to pleomorphic in shape [3]. It's one of the four genera (alpha-CoV, beta-CoV, gamma-CoV and delta-CoV [7]. Gamma-CoV are enveloped, positive-strand RNA viruses that infect birds, mammals and humans having the largest of all RNA virus genomes (27-31kb) in size, capped, and polyadenylated. It is largest among positive-sense single-stranded RNA viruses and the leader RNA (65-89 bp) at the 5' end of the genome is also present at the end of each sub-genomic RNAs [7]. Virions constitute spike (S), envelope (E), membrane (M) and nucleocapsid (N) structural proteins [8]. The S glycoprotein is a trimer composed of subunits S1 and S2 having roughly 520 and 625 amino acids, subsequently. The viral binding domain is located on S1 subunit of S protein [9]. The IBV lacking S1 subunit is not virulent nor can cause much mortality [10]. IBV serotypes have unique variability in S1 subunit sequence of S protein, *i.e.*, some serotypes differ 20-25%, while others up to 50% [11]. Gamma-CoV attaches and enters the host cell by its spikes that is a glycoprotein in nature, 20 nm in length [8]. These spikes give IB virus crown shape, hence called coronavirus. E protein is minor essential protein and its function is the aggregation of the virus [12]. Glycoprotein M springs around viral coating thrice outside with a portion of NH3. Phosphoprotein N envelops the viral RNA genome to make circular nucleocapsid in the virus and interplay with M and E proteins for virus aggregation (Fig. 1) [3].

Why infectious bronchitis virus is so difficult to control?

Since its discovery in 1930, infectious bronchitis remained a continuous challenge for the poultry industry causing huge economic losses in form of mortality and welfare of birds (broilers, layers, pullets, rosters, breeders). From the aspect of emerging and re-emerging diseases around the globe in poultry production, infectious bronchitis remains centered. There are several reasons for its lethality. First and foremost is that its genetic material is RNA [3]. As they are RNA based viruses, their control is most challenging. If a proper vaccine is available at the specific timing of disease eruption, production flock can be protected but if there is a lack in its strain or variant diagnoses, a certain lapse in vaccine availability or vaccine application will cause virus eruption and huge economic loss. The second major reason is poor diagnosis. As mentioned before, until a specific strain or variant of infectious bronchitis is identified causing disease eruption and other related issues, vaccination is useless. So, the first thing is to identify the strain and plan the vaccination schedule accordingly. The third potential reason is poor, improper, or unprofessional vaccination jobs. In such cases, among a flock, birds that remained unvaccinated become amplifiers of viruses causing infectious bronchitis virus to spread. Thumb rule against disease protection is surveillance of commercial poultry birds, *i.e.*, maintaining routine check and balance against disease and especially in case of infectious bronchitis, it is unavoidable. Geographical location and higher concentration of poultry farms and poultry production units cause birds prone to infectious diseases and infectious bronchitis attacks. Poultry producers should avoid all contact with other sources of poultry and markets [13]. Recent studies depict that a new variant of infectious bronchitis follows certain routes in the area having a high concentration of poultry birds. Being an airborne virus, it can easily float from one farm to another. Lack or ignoring basic poultry husbandry practices stands an important reason for the infectious bronchitis eruption. Vaccination is a powerful tool to ensure poultry health but without following a holistic approach, *e.g.*, biosecurity measures and sublime management and combing all husbandry practices, IBV control becomes critical. The immune status of birds also plays important role in infectious

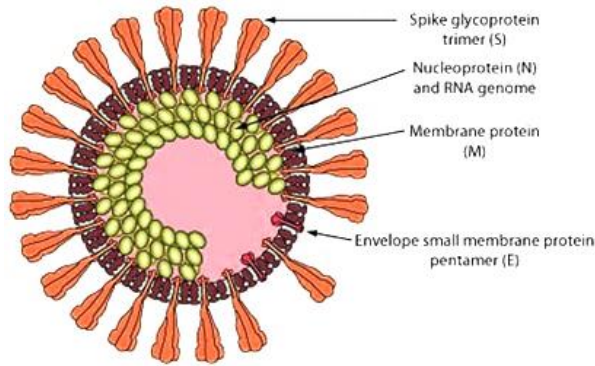


Fig. 1 Gamma coronavirus: shape and structure [88].

bronchitis prevalence. Broilers, layers and breeders are prone to viral diseases and if they get these viruses, it's very easy for bacteria and dust particles to penetrate deep into tissues of the respiratory tract. Flock mortality and morbidity losses are also high in infectious bronchitis because other opportunistic secondary pathogens like *Escherichia coli*, pneumonia, mycoplasma, airsacculitis, peritonitis and the false layer syndrome take advantage of infection [14, 15].

Clinical Signs

Infectious bronchitis causes primary pathogen in the upper respiratory tract, causing gasping, watery eyes, mucus in the trachea and coughing [14]. Primary infection of infectious bronchitis is a gateway to secondary bacterial infections as mentioned above. Poultry birds of all ages are affected by IBV. It affects embryonating eggs, resulting in crippled, dwarf and infected embryos [3]. Body weight of bird drops due to reduced feed intake. External and internal egg quality decreases, showing deformed and inferior eggshell, watery albumin [16]. Kidneys are severely affected having kidney lesions only with some types of virus, *e.g.*, TW-like IBV strain GD [17]. Tubules observed in swollen, pale kidneys, liver, heart [18] and ureters expanded with urates [3]. Along with other organs, the reproductive system of birds also gets affected both in hens and cockerels. Replication of IBV in the oviduct causes disruption in calcium deposition in eggshells, resulting in deformed eggs [18]. In pullets and hens, cysts of varying sizes, protruding in the right oviduct, condensed oviduct, fibrin clots in magnum and uterus and yolk peritonitis in infected birds occurred [16]. Zhong et al. observed epithelial necrosis and desquamation, epithelial edema, follicular edema, degeneration of vacuole, congestion and absence of lumen and epithelium

cells [19]. Deleterious effects of infectious bronchitis on the reproductive health of roosters and cockerels need serious attention and are lacking. Testicular histopathological findings depicted micro-bleeds, tubular degeneration, focal necrosis and vascular congestion [20]. In some reports, the live attenuated and killed IBV vaccine increased the incidence of epididymal stones, decreasing sperm and testosterone concentration [21, 22]. In addition, the use of a live attenuated IBV vaccine increases the incidence of epididymal stones in roosters, resulting in decreased sperm production and decreased serum testosterone concentration [23]. Reproductive tract disruption in male and female birds largely depends on viral load, infection period and host specific factors. These deleterious effects on the reproductive tract of birds results in poor hatchability, low breeding values and infected semen in production flocks [24].

Association of COVID-19 with infectious bronchitis

COVID-19 is caused by SARS-CoV-2 that belongs to the beta-CoV group [25]. Viruses in the beta-CoV group don't cause infection in poultry, while avian CoV, grouped in Gamma-coronavirus, do not affect humans [26]. According to de Wit and Cook [27], gamma- and delta-CoV are found in birds. There is no evidence that SARS-CoV-2 will affect poultry [28-29].

Transmission

Being an air-born virus, it's extremely contagious and spreads among birds in a flock [30]. Animal husbandry practices should be strictly followed, *i.e.*, biosecurity, proper and timely vaccination, avoiding outsiders and wandering birds, *e.g.*, goose, and swans, poultry house disinfection and keeping ammonia levels in control. Within 24 hours post-infection, infectious bronchitis infected chickens begin to show respiratory signs of disease in experimentally challenged birds, but natural infection can take longer for signs. Infectious bronchitis is an upper respiratory tract disease and high concentration of viruses in the respiratory tract causes aerosol droplets to transmit viruses making viral worse. During the initial 3-5 days of infectious bronchitis attack, there is the highest concentration of IBV in the trachea [3]. If birds are properly vaccinated and protective measures are adopted timely, IBV load in the trachea decreases

drastically [31] reducing secondary pathogen attack. Viruses are also excreted in uric acid from the kidneys and in droppings [27]. Contaminated and wet litter not only causes viral transmission from flock to flock but sticks with caretaker personnel shoes and clothing [3] inviting other viral, bacterial and fungal pathogens. Fomites, a higher concentration of poultry birds in an area and migratory birds like geese, pigeons, wander, vultures can be potential sources of IBV breakout. Some closely related IBV strains were identified in wild birds [32]. Vertical transmission of IBV in a day-old chicks was observed whose mothers (hens) were experimentally infected with IBV [33]. IBV was also isolated from semen of male breeder flock confirming the possibility of IBV infection in the oviduct of the vulnerable hen [34].

Control strategies

Effective diagnosis and vaccination: a sublime tool against infectious bronchitis

Vaccination is a pillar in commercial pullet health and welfare [35]. IBV has limited proofreading capability, *i.e.*, it replicates rapidly and makes changes that are difficult to cope up. Further, these alterations pass on generations where IBV replicates more abruptly even in the phase of vaccination [36, 37]. As mentioned before, to ensure flock health, we have to adopt a holistic approach, among which, vaccination stands centered [30, 34]. Extensive IBV vaccination doesn't ensure flock protection unless the right combination of vaccines and diagnostics are practiced, *e.g.*, in Saudi Arabia, persistent outbreaks of IBV were observed in broiler and layer flocks despite extensive vaccination [38]. The problem with the right application of vaccines at the right time is that IBV has hundreds of genetic variants and the solution is to use a combination of vaccines that can give a broader immune response against circulating field viruses unless we have homologous vaccine [39]. IBV can occur in various serotypes that differ in their surface proteins or spikes. Some serotypes show marked differences, but others have similarities in their structure and are classified into groups. Certain serotypes are used as a vaccine because they induce immunity against more than one serotype called as protectotypes, and this concept is called as heterologous vaccination [40-42]. To find the right combination of vaccines against IBV, the virus neutralization study gives more effective vaccine

indication, but it is time- and cost-consuming methodology. Another method is genetic testing in which spike protein sequences elaborates closely related specific IBV strains, on its basis, vaccination plan can be decided accordingly. The IBV modified live vaccines are more effective than inactivated vaccines as their application in the hatchery is more efficient and easier to monitor [43, 44]. In both hatchery and field, live attenuated vaccines are more cost and time effective. In layer birds, attenuated vaccines can be applied between 4-6 weeks depending on IBV virulence [39].

Being enveloped virus, IBV vaccine strains are fragile and easily affected by shearing forces and temperature. An important aspect is that IBV vaccine need to keep cool before it warms at room temperature to attain the full titer of the vaccine. IBV attack is predominant in winter, so major mistake made by poultry producers at farms is to cut vaccine dose to save some money and to prevent vaccine reactions because in summer IBV doesn't seem much predominant resulting in inadequate immunity. Therefore, the birds remain susceptible to disease, allowing field viruses to replicate which is termed as genetic drift. These field viruses in under-vaccinated or poor-vaccinated birds replicate over time, resulting in the emergence of new strains of IBV. The volume of vaccine also matters, *e.g.*, in America, IBV vaccine dose of 7 ml/100 chicks was practiced for a long time that is insufficient to immunize chicks. The droplet size of vaccine also matters, so, the recommendation is to use fine droplets and the dose should be 14 ml/100 chicks to get optimum protection against IBV. A fixed pressure of 100-300 microns for 10-15 minutes over chicks or 30-40 cm above chick's boxes is recommended. Chicks boxes should be allowed to dry 15 minutes before dispatch from the hatchery [35]. In layers and breeders, the recommendation is to mix the IBV vaccine in drinking water. Droplet behavior, size, water quality, climatic conditions, house layout, site (hatchery/farm), vaccinators experience, equipment (type of sprayer and sanitized) affect successful spray of IBV vaccine [35]. The accurate amount and timing of vaccine ensure broad spectrum flock protection [45-47].

Molecular diagnostics

Real-time PCR is a powerful tool to diagnose IBV in comparison with other closely related diseases. Certain RT-PCR tests can also distinguish types of viruses, *i.e.*, they are vaccine-related or field-related

which helps to match their genetic sequences for better vaccination plans. Avian mycoplasmosis, infectious laryngotracheitis, Newcastle disease and avian influenza have similar clinical signs like infectious bronchitis, so preventive measures should be taken to differentiate appropriately [48-54]. Now vaccine-related viruses and pathogenic viruses can be screened. Hemagglutination inhibition, virus isolation, virus neutralization test and enzyme-linked immunosorbent assay (ELISA) are also in practice for virus detection and confirmation [3,11, 55-57]. Single-step-real-time-RT-LAMP (loop-mediated isothermal amplification assay) serves as a cost-effective molecular and semi-quantitative way for IBV [58].

Microarray analysis of dendritic cells

Dendritic cells are accessory cells (also called antigenic presenting cells) of the immune system that are responsible for processing antigens and presenting them to T cells [59]. Virus neutralization, recognition, messages to and from between innate and adaptive immune systems are their vital functions [60]. Avian dendritic cells can induce both acquired and innate immunity and are present in primary and secondary lymphoid organs [59]. Maturation of dendritic cells is inevitable in immunity development and invading viruses have a plan of action to target this process [61, 62]. Among non-coding RNA's, miRNA's are not only key regulators of the immune system [63, 64], but also shape defensive mechanisms against invading viruses [65, 66]. A novel mechanism was elucidated to reveal an interaction between avian dendritic cells and IBV in which gga-miR-21 can regulate the defense mechanism against viruses in chickens. This method also provides defensive or therapeutic strategies against IBV infection [59].

Identification of high affinity Gallus gallus aminopeptidase (gAPN)

Gallus gallus aminopeptidase (gAPN) is a zinc-dependent metalloproteinase of M1 family [67, 68] having a molecular weight ~180 KDa [69]. In IBV genome, S1 protein facilitates attachment to viral receptors and entry into host cells [70, 71]. *In vitro* and *in vivo* results showed binding of gAPN high-affinity phages to IBV S1 protein polyclonal antibody and IBV neutralizing antibodies production, respectively [72], having the ability to reduce IBV infection. However, the shortcoming is that the inhibition rates of gAPN high-affinity ligand to IBV infection were <80%. New methods

are required to synthesize optimized peptides to improve antiviral activities, enhance phage immunogenicity or homologous vaccine development for various IBV receptors [72]. *In vivo*, IBV infection is highly mediated by DC-SIGN and L-SIGN distribution in hematopoietic cells, *e.g.*, macrophages that can propagate IBV to other organs [73]. However, the intricacy of lectin-virus in IBV receptor complex needed to be unveiled.

Haemagglutinin-sialosides interaction

Sialic acid-containing carbohydrates or sialosides play a vital role in various biological events, including bacterial and viral infections [74]. IBVs are emerging and re-emerging viruses having hundreds of serotypes that vary in S1 subunit sequences on S protein. Due to high diversity, gamma-CoV also include members that lack sialic acid binding activity [74]. All IBV serotypes having sialic acid do not bind to glycans with the same strength depicting that sialic acid binding activity is a conserved feature of IBV [9]. Primary chicken kidney cells became resistant to two IBV strains (Baudette and M41) after removal of sialic acid from the cell surface by neuraminidase treatment [28]. Sialic acid isomers α 2,3-linkages bind on the epithelial cells of host chickens and gut of waterfowl [74]. If authentic sialoside for the cognate receptor is identified, more information from haemagglutinin-sialoside interaction will postulate better vaccine development to prevent this binding, thus blocking host infection [74]. However, it's not a simple task and intricacy needed to be determined.

Immune responses from major histocompatibility complex (MHC) of congenic chicken lines

Although there are similarities between mammalian and avian immune systems, still avian species have distinct mechanisms and immunological structures [75]. Exploring the immune response of chickens is a new domain against IBV control and resistance strategy. It's not merely bringing about genetics to chickens because some genetic traits among chicken breeds have less productive efficiency. Some researchers are trying to explore genetic lines in chickens that are resistant to IBV. Chicken breeding strategies as a selection tool for developing disease-resistant varieties may improve the overall efficiency of poultry production [76]. Key components in innate immune response and adaptive immune response

will give substrate to study and come up with new strategies that are different from vaccines. Innate and adaptive immune responses are influenced by genetics [76]. Exploring genetic resistance and innate immunity can assist in developing preventive strategies against IBV [77]. There would be variability of responses to IBV and should come up with different preventive strategies. In this specific domain, birds with different major histocompatibility complex (MHC) but having the same genetic and biological background are selected, so if such birds are challenged against IBV, they will have different responses because of variations in their major histocompatibility complexes (MHCs). MHC locus B has been proven to be resistant to various pathogens, including IBV [77-81]. MHC haplotypes B2 and B19 were resistant and susceptible to IBV M41 and ArkDPI challenges, respectively [82]. Strong linkages for various poultry diseases have been studied between MHC and resistance to infectious diseases [77]. Components of the innate response to IBV might provide a better understanding of Newcastle disease and avian influenza. Resistant genes (MHC-B locus genes, MX1, OASL and IFITM3) can assist to postulate targets for future resistant genetic crosslinks to IBV [83]. In another *in vivo* experiment, MHC congenic chicken lines 331/B2 and 335/B19 were challenged with IBV strain M41 and ArkDPI in the trachea and after 60 hours, cytokines (IFN β , IL-1 β , IL-6, IL-10) genes expressions elucidated better performance of chicken congenic line 35/B19 showing more resistant against IBV [84]. While working on specific chicken lines of major histocompatibility complex (MHC), it should be kept in mind that these genetic lines are exclusively selected for their immune responses and traits that are inversely proportional to various production characteristics [85, 86], *e.g.*, meanwhile seeing immunity responses, body weight is not ideal trait while working on specific layer chickens using MHC, but bodyweight is an ideal trait in case of broilers [87].

Serology

Antibody-based detection methods are used to check IBV or immunological response in poultry flocks. Serological tests, including ELISA, agar gel precipitation (AGP), virus neutralization (VN) and hemagglutination inhibition are practiced. ELISA tests are routinely practiced in serological monitoring due to high efficiency and cost-effectiveness. For accurate results, baseline values

should be based on local geographical location because antibody titers depend on various factors, *e.g.*, vaccination program, breed, sampling age, etc. Virus neutralization and hemagglutination inhibition are useful to check the specific response of vaccine and field serotypes because they can identify serotype-specific antibodies. However, hemagglutination inhibition is considered a better method for serotyping purposes. Virus neutralization is considered laborious for its routine monitoring.

Zinc and Manganese supplementation in feed

Trace minerals zinc (Zn) and manganese (Mn) have a major influence on immune responses. In the respiratory tract, Zn is an important regulator of the immune response [87]. They interfere with phagocytosis, cytokines production, inflammation, maturation, and differentiation of immune cells, *e.g.*, macrophages. Zn and Mn are used as feed supplements of birds having the same genetic origin and their major histocompatibility (MHC) is linked to relative resistance or susceptibility to infectious diseases in general [82]. In a disease outbreak, airborne pathogens lower accessibility of Zn by descending presence of Zn carriers, which ultimately malfunctions immune response. *In vitro*, Zn blocks CoV RNA replication. Positive effects of amino acid bound Zn and Mn provide a gateway to other amino-acid bound minerals to improve their bioavailability in MHC congenic chicken lines [87]. Thus, these supplements have not been shown to protect specifically against IBV and postulates further investigation.

Conclusion and challenges

In the case of IBV control, everything is retrospective, there are techniques to know what evolutionary IBV takes and how rapidly it changed and be prepared for tomorrow, but we cannot predict future outbreaks. Despite adopting various preventive measures against IBV, there is still a long way to go. Worldwide, poultry industry is moving towards more judicious use of antibiotics and in most cases eliminating them, a new challenge for poultry industry. Lapse in diagnostic and preventive infrastructure also remains a hurdle in IBV control worldwide. Prevention is better than cure in the case of IBV, "eye of a storm". Academia and poultry industry needs practically more effective collaborations to develop better, effective, robust, and cost-effective methods and technologies to combat IBV.

Conflict of Interest

The authors declare that this article's content has no conflict of interest.

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