Review Article

Infectious Bronchitis: A Challenge for the Global Poultry Industry

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Abstract | Infectious bronchitis caused by infectious bronchitis virus (IBV) is an acute, highly contagious and economically important disease of poultry. The disease has remained a serious threat to the world poultry industry since its discovery in 1931. The virus mainly affects the respiratory, renal, and reproductive systems. Multiple serotypes of IBV have emerged due to the high rate of mutation and genetic recombination, which has made it difficult to control the disease. Certain serotypes have disappeared because of the availability and use of vaccines but new serotypes have emerged. A regular watch on the disease including virus variation, prevalence, pathogenesis and development of diagnostic tools are important for the formulation of effective prevention and control programs. This review deals with the global prevalence of IBV along with its pathogenesis, diagnosis, and control. In summary, we have discovered that measures to combat infectious bronchitis is not fully effective and the continued attention of the scientific community and funding agencies can help protect the global poultry industry from this challenge.

Received | June 22, 2021; **Accepted** | December 5, 2022; **Published** | May 04, 2023
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Citation | Khan, S.Z., M. Waqas, and S.M. Goyal. 2023. Infectious bronchitis: A challenge for the global poultry industry. *Sarhad Journal of Agriculture,* 39(2): 416-432.
DOI | https://dx.doi.org/10.17582/journal.sja/2023/39.2.416.432
Keywords | Control, Diagnosis, Infectious bronchitis, Pathogenesis and prevalence

Introduction

Infectious bronchitis (IB) is an acute, extremely contagious and economically important disease of poultry (Arshad, 2006). The origin of the disease is North Dakota, USA (Schalk and Hawn, 1931). It has remained a severe risk to the world poultry production since its detection. The causative agent of this disease is infectious bronchitis virus (IBV) which belongs to the genus gamma coronavirus under the family *Coronaviridae*. The virus is distributed throughout the world (Cavanagh, 2007) and is responsible for austere fiscal damages to the poultry business (Liu *et al*., 2005; Chen *et al*., 2013). IBV is an extremely contagious virus and spreads very rapidly. The IBV spreads rapidly through direct contact, fomites, and aerosols. After entering via the conjunctiva or nasal cavity, the is 18-36 hours. Poor hygienic conditions at the farm, improper ventilation, and overpopulation of birds are some predisposing factors for IBV infection (Cavanagh and Gelb, 2008). The virus is heat sensitive and is killed within 15 min at 56°C. The virus is also sensitive to certain disinfectants, solvents, and alkalis (McKinley *et al*., 2011). No evidence exists to

indicate if IBV is zoonotic in nature although workers engaged with poultry flocks do possess neutralizing antibodies (Kapikian *et al*., 1969). Poultry of all ages are prone to IBV infection, which mainly affects respiratory, reproductive and renal systems. Infected birds show signs of respiratory discomfort which includes sneezing, coughing. tracheal rales and nasal discharge (Kanwal *et al*., 2018). The virus may cause renal failure and infection of the reproductive system which leads to the reduced fertility, decline in egg laying and poor quality of eggshell (Seidek, 2010). The growth performance of broilers is also affected by IBV; it causes poor weight gain due to reduced feed consumption (Cavanagh, 2007).

Infectious bronchitis is often related to secondary bacterial infections with *Escherichia coli* and *Mycoplasma* spp. resulting in increased condemnation rates at processing (Cavanagh, 2007).

Although vaccination is considered to be the most effective method of combating the disease is (Meeusen *et al*., 2007), however there are some factors that challenge the effectiveness of vaccines this includes the development of novel serotypes that exhibit slight or no cross-protection (De Wit, 2000). It is noteworthy that certain serotypes, against which vaccination is usually practiced, might have disappeared but new variants have taken their place. This makes it essential that new vaccines be developed against new variants (Meeusen *et al*., 2007). Presently live attenuated and killed vaccines are used for vaccination against IBV. These vaccines include IBV strains originating from the USA and the Netherlands (including M41, Ma5, Ark, Conn, H52 and H120), etc. However, birds show poor immune response against these vaccines because birds are not fully protected against Pakistani local variants (Rafique *et al*., 2018). It is also notable that live attenuated vaccine has been implicated in the development of new pathogenic IBV strains (McKinley *et al*., 2011).

Efforts are needed to understand the disease and its emerging strains for developing newer diagnostic techniques, effective vaccines and for adopting appropriate control measures (Cavanagh, 2007; Bande *et al*., 2017). This review is an effort to attract the attention of world poultry experts to combating this economically important disease.

Prevalence of IBV in various countries Pakistan

Muneer *et al*. (1999) tested 2185 blood samples from 110 flocks from different geographical regions of Pakistan. The outcomes of the hemagglutination-inhibition (HI) test showed the presence of antibodies against various IBV strains including JMK, Massachusetts-41 (M-41), Arkansas, D-1466 and D-274. In another study, Hussain *et al*. (2005) tested 360 serum samples from 21 unvaccinated broiler farms in southern Punjab and found an overall prevalence of 2.22% with significantly higher incidence (5.36%) in 1-2 week-old broilers. Mustafa and Ali (2005) reported a prevalence of 7% in the Fayoumi breed in Sheikhupura. Ahmed *et al*. (2007) screened serum samples from 16 layers and 9 broiler flocks from Faisalabad, Pakistan. They found 100% layers and 66% broiler flocks as seropositive with the highest positive for M-41 antibodies followed by D-274, D1466, and 4-41 IBV strains, respectively. In a study of broilers and layers in 360 poultry farms in the Khushab district of Pakistan, the prevalence was 1.59% (Abbas *et al*., 2015). Molecular characterization of an IBV isolated from a suspected bird in commercial poultry at Attock, Pakistan was conducted by Rafique *et al*. (2018). They observed that bulk use of live IBV vaccine strains of diverse origin has resulted in the emergence of a variety of new strains through mutation and genetic recombination. Rahim *et al*. (2018) screened 500 serum samples from six breeder and broiler flocks by HAI test and found seroconversion against serotypes D-1466 and D-274 in breeder poultry in Abbottabad and Qalandarabad. Breeder farms at Sihala (Islamabad) and Multan also showed seroconversion against IBV serotype H-120 and 4/91, while in Jumma Bazar Broilers, seroconversion against serotype M-41 was observed. These studies concluded that numerous IBV strains were circulating in commercial poultry production units in Pakistan however further work is needed to isolate and biologically characterize the prevailing IBV strains to formulate an effective vaccine against IBV.

Iran

Massachusetts strain is the most prevalent IBV serotype in Iran. Other prevailing strains in the country are European D274 and 4/91 (793/B)-like strains) (Seyfi *et al*., 2002). Later, Seyfi *et al*. (2004) reported the 793/B serotype to be more prevalent in broilers than Massachusetts serotype. In another study, 150 flocks were tested from 1999-2004, of which 52.2% were positive for 793/B serotype, 16.6% for Massachusetts type and 30.5% had a dual infection

(Selim *et al*., 2013). Shokri *et al*. (2018) reported a seroprevalence of 54.5% in backyard chickens; serotypes like Variant 2, 793/B, and QX were found. The high homology of the detected genotypes was observed with IBV strains infecting pullets, layers and broilers in Iran. In another study, Boroomand *et al*. (2018) reported 48% prevalence in broilers. The phylogenetic analysis of the isolates revealed QX-like viruses such as PCR Lab/06/2012 (Iran), QX, HC9, HC10, CK/CH/JS/YC11-1, CK/CH/GX/NN11-1 CK/CH/JS/2010/13, CK/CH/JS/2011/2 (China), QX/SGK-21, and QX/SGK-11 (Iraq) with nucleotide similarity around 99%.

China

Feng *et al*. (2017) studied the incidence of IBV in China during 2013-2015. They isolated 206 strains of IBV from poultry showing signs of infectious bronchitis. They confirmed seven different genotypes flowing in commercial poultry farmhouses in Southern part of China. The most prevalent genotypes were QX-type, TW I-type, and 4/91 type, which were about 88.8% of the total isolated strains. The QX- type genotype was the most abundant (46.7%) and was present in all the surveyed provinces. During 2004-2012, the major genotype of IBV in China was the QX-type (Sigrist, 2012; Luo *et al*., 2012; Feng *et al*., 2014). Later, a slight decline was observed in QX-type because of the use of QX-type attenuated vaccine (Huo *et al*., 2016; Feng *et al*., 2015). Recently, TW I-type has emerged as the most prevalent genotype of IBV in China (Feng *et al*., 2017).

India

According to the (Patel *et al*., 2015) Massachusetts strain was found to be the most predominant IBV stain in India. Bayry *et al*. (2005) defined the rise of a nephron pathogenic IBV with a new genotype in India. The isolate showed a very high variation in sequence as compared to the reference strain. The maximum genetic identity was witnessed with strain 6/82 (68%) and the minimum with strain Mex/1765/99 (34.3%). Nephopathogenic strains were also isolated from poultry in Anand, Gujarat (Bayry *et al*., 2005; Parveen *et al*., 2018; Patel *et al*., 2015; Sumi *et al*., 2012). Patel *et al*. (2015) reported the existence of IBV isolates in various states of India such as Tamil Nadu, Assam, Andhra Pradesh, Maharashtra, Uttar Pradesh and Orissa. In India, IBV strains typically belong to genotype-I, lineages 1 and 24, and serotype Massachusetts (Valastro *et al*., 2016). Nevertheless, very less information is available about the whole genomic sequences of IBV isolated from various parts of the country.

Africa

According to the Khataby *et al*. (2016) the most common respiratory disease of Poultry in Africa is Infectious Bronchitis. Ayim-Akonor *et al*. (2013) discovered IBV in Ghana in poultry. A variety of indigenous variants have been identified in Africa along with the preexisting widespread vaccine serotypes e.g., Massachusetts and 793/B (de Wit *et al*., 2011). In a current study, Ayim-Akonor *et al*. (2018) obtained sera from 440 broilers and layers District Ga-East and found 85.5% to be positive. Recently, in 2010 and 2013, more serotypes of IBV were identified in the southern and central areas of Morocco. These serotypes included IBV/Morocco/01, IBV/Morocco/30, and IBV/Morocco/38, Italy 02, which is one of the common strains in the Europe, is the second most prevalent genotype in Morocco (Dolz *et al*., 2012; Fellahi *et al*., 2015a, b). In South Africa, Mass strain is the principal serotype while CK/ZA/2034/99, K/ZA/2281/01, 793/B and QX-like, are also prevalent as reported by (Knoetze *et al*., 2014). Toffan *et al*. (2011, 2013) unveiled two variants of IBV i.e. The MJT1 and MJT2 in non-vaccinated poultry in the Beit Bridge region, adjoining Zimbabwe. In Sudan, four serotypes were identified e.g., M114/2000, K170/2000, K110/2000, and K158/2000. The first of these belongs to the European 4/91 subgroup while K110/2000 is similar to the Massachusetts type (Ballal *et al*.,2005).

Canada

Canadian IBV strains isolated from an outbreak closely resembled the Massachusetts vaccine (M41) and the Connecticut strains. In Ontario, IBV-ON1 and IBV-ON4 have been reported; the former distresses the respiratory system while the latter is associated with nephritis. It is notable that chickens vaccinated against Mass serotype also showed protection against the Ontario strains (Grgić *et al*., 2009). Later, nine other serotypes were identified, which were divided into four groups including the Canadian variant (strain Qu-mv), classic (vaccine-like viruses, Conn and Mass), US variant-like virus strains (California 1734/04, California 99, CU_82792, Pennsylvania 1220/98 and Pennsylvania Wolf/98), and non-Canadian, non-US or European strains (793/B strain) (Martin *et al*., 2014).

America

The first ever case of IB was detected in 1930 in the USA (Schalk and Hawn, 1931). After that several strains of IBV were discovered of which the Massachusetts strain is the most common. Other serotypes identified in the USA are Connecticut, Arkansas, Delaware and SE17 (Jackwood *et al*., 2005). Since the 1980s, most of the respiratory strains have been reported in broilers in central California. These serotypes were distinctive in their matrix protein polymorphism and were diverse from the Conn, Ark-99, and Mass strains. A nephron pathogenic strain of IBV also known as CAL99 was reported in 1999. After that another three variants of IBV i.e. [CA1737/04, CA557/03 and CA706/03] were reported (Jackwood *et al*., 2007). Mondal *et al*. (2013) in their study stated that twelve strains of IBV were isolated in the 1960s. Out of these twelve strains, seven belonged to Mass, five belonged to SE17 and one was similar to Conn genotype.

Australia

A majority of the Australian IBV strains cause nephritis while a few strains are involved in respiratory disease. The nephro-pathological strains are of more interest because they cause clinical nephritis and are responsible for death in poultry (Ignjatovic *et al*., 2002). Two groups of IBV are recognized in Australia. Group 1 includes N1/62, N3/62, N9/74, N2/75, Vic S, and V5/90. They have 80.7-98.3% similarity in their amino acid sequence with Vic S. The second group of IBV in Australia includes N1/88, Q3/88, andV18/91. This group is less pathogenic and does not cause mortality. Recently, another group (Chicken/Australia/N2/04) has been reported, which showed little similarity to the former two groups. However, it had more resemblance with D1466 and DE072 strains from the Netherland and America, respectively (Bande *et al*., 2017).

Europe

De-Witt *et al*. (2018) investigated the prevalence of IBV in various countries of Europe including Poland, Holland, Spain, Ireland, Portugal, Germany, Greece, and United Kingdom. A total of 234 samples was tested from which 10 different genotypes of IBV were seen. The most common genotype was 793B which was tailed by QX, Massachusetts, and Xindadi-like strains. Other strains detected were Ark, D274, D1466, Q1, B1648, and Itlay-02. Another study reported 793B as the most predominant serotype of IBV in Western Europe followed by H120, IBM, M41, Mass type, and a variant close in resemblance to Chinese QX (Worthington *et al*., 2008). According to Bande *et al*. (2017), the QX-like strain of IBV appeared as the utmost threatening strain of IBV in Europe. Other IBV serotypes like QX, D274-like and 4/91-like were recently identified in Finland (Pohjola *et al*., 2014). The prevalence of QX-like serotypes were reported in Scotland, Italy, Poland, the Netherlands, Spain, the UK, Sweden and Slovenia (Worthington *et al*., 2008; Krapez *et al*., 2011; Valastro *et al*., 2010; Abro *et al*., 2012; Domańska-Blicharz *et al*., 2007).

The Middle East

In Middle East, strains of IBV vary from one country to another. The DY12-2-like, a Chinese like recombinant virus was a prominent strain in the region (Seger *et al*., 2016). A study was conducted during 2009-2014 in seven Middle Eastern countries. A total of 461 samples were tested out of which 363 came out positive by RT-PCR. The serotypes included 793B (43.66%), IS/1494/06 (18.31%), Massachusetts (12.96%), IS/885/00 (11.27%), Q1 (11.27%), and D274 (2.25%). The most prevalent genotype was 793B, probably because it is widely used as a vaccine in the Middle East Countries (Ganapathy *et al*., 2015).

Egypt

In Egypt, IB was first reported in early 1950s (Ahmed, 1954). Although Mass type vaccine was used extensively, the disease continued and became a major problem in Egypt. In 2002, Egypt/Beni-Suef/01 was discovered in Egypt (Abdel-Moneim *et al*., 2002). Although the genotype was exceptional to the country, it closely resembled the nephron-pathogenic strains of Israel (IS/1494/06 and IS/720/99; Meir *et al*., 2004) and, when injected in chickens, caused critical respiratory and kidney disorders (Abdel-Moneim *et al*., 2005). Another strain (Egypt/F/03) was reported in 2006, which was nephro-pathologic in nature and was similar to the Dutch strain D3128, the Israel IBV variant, and the Massachusetts variant (Abdel-Moneim *et al*., 2006). Other Five variants of IBV were reported in 2011; these included Ck/Eg/BSU-1/2011, Ck/ Eg/BSU-5/2011 (which clustered with Egypt/Beni-Suef/01 and Israeli IS/1494/06) Ck/Eg/BSU-2/2011, Ck/Eg/BSU-3/2011 and Ck/Eg/BSU-4/2011. These variants were different other previously identified Egyptian variants or serotypes (Abdel-Moneim *et al*., 2012). Later studies revealed that Egyptian variants were highly diverse and were

different from classical variants (Zanaty *et al*., 2016a).

Iraq

In 2014-2015, four groups of IBV were described in Iraq including group I: variant 2 [IS/1494-like], group II: 793/B-like, group III: QX-like, and group IV: DY12-2-like genotypes of IBV. Among them, a group I was the most prevalent (Bande *et al*., 2017). The 793/B genotype IBV also exists in Sulaimani, Iraq. Vaccination against 793/B and Massachusetts is routinely done. A novel IBV variant (Sul/01/09) was also found in poultry farmhouses and is said to be unique from other existing variants of IBV in this country (Mahmood *et al*., 2011).

Etiology

This disease is caused by IBV, a corona virus. The structural proteins of IBV consists of four proteins. These include the matrix M, the nucleocapsid N, the envelope E and the spike S glycoprotein. These proteins play diverse roles in replication, clinical disease and viral attachment. The S protein is responsible for the adsorption and fusion of virus with the cell membrane for releasing the virus RNA in the host cell's cytoplasm. This S protein is further subdivided into two subunits, S1 (amino-terminal component) and S2 (carboxy-terminal component). The specificity and pathogenicity of IBV depend upon this spike or S protein (Zeng *et al*., 2006).

The serotyping of IBV is based on differences in their S1 spike protein. Most of the serotypes have more than 20-25% differences in S1 protein while some show more than a 50% difference, which makes cross-protection of these serotypes very poor (Cavanagh *et al*., 2007). The variability of S1 protein is either due to the absence of proofreading of RNA-dependent RNA polymerase that presents mutation in the viral genome during replication or due to specific template switching mechanism used by IBV that leads to genetic recombination (Pasternak *et al*., 2006). The M protein is a transmembrane protein and is the most abundant. The M protein interact with viral ribonucleic-capsid and spike glycoprotein and plays a vital role in virus assembly (Bande *et al*., 2015). The protein E of IBV contains highly hydrophobic transmembrane N-terminal and cytoplasmic C-terminal domains. The E protein is associated with envelope formation, viral assembly, budding, apoptosis, and ion channel activity. This protein resides in the Golgi complex of the IBV-infected cells (Wilson *et al*., 2006). The N protein of IBV contains 409 phosphorylated amino acids that are highly conserved between 238 and 293 amino acid residues. The N protein helps in viral genome transcription, replication, translation and packaging, through binding with the genomic RNA, to form a helical ribonucleoprotein complex (Jayaram *et al*., 2005).

Pathogenesis

The severity and pathogenicity of the disease depends upon the organ or system involved (Cavanagh and Gelb, 2008). It also depends upon the bird's immune power, age and the pathogenic strength of the IBV serotype (Kuldeep *et al*., 2014). The IBV has the potential to multiply in a wide range of body systems. These systems are Respiratory, Urogenital and Digestive systems (Boroomand *et al*., 2012). Although it primarily affects the respiratory system (Cavanagh, 2007). After infection, it replicates in epithelial tissues of respiratory tract and then in kidneys, bursa and gonads (Cavanagh, 2007).

The virus enters via the respiratory route and initially multiplies in the upper respiratory tract after which viremia develops. Proliferation in other organs like kidneys and oviducts can also occur. The Incubation period of the virus ranges from 18-36 hours depending upon the route of entry (Cavanagh and Gelb, 2008). Following the acute phase, systemic infection develops in various organs where the virus continues to multiply and be excreted. The IBV is epitheliotropic in nature and proliferates in the epithelial tissues of many organs including the oviduct, kidneys, and respiratory tract. It also proliferates in the alimentary canal but shows little pathological or clinical signs (Ignjatovic *et al*., 2002). In the respiratory form of IBV, serous, caseous, or catarrhal exudates are observed in the nostrils, sinuses, and trachea. The Lower part of the trachea and bronchi of young birds are filled with caseous plugs. There is thickening and opacity of the air sacs. Pneumatic focal areas may also appear (Abdel-Moneim *et al*., 2005).

Gross lesions in kidneys are uncommon but some microscopic pathological changes can be observed in cases of nephritis. Kidneys become pale and swollen with ureters and tubules inflated due to accumulation of urates (Abdel-Moneim *et al*., 2005). In the reproductive system, the most affected organ is the middle third of the oviduct (Abdel-Moneim *et al*., 2005). Lesions in the oviduct lead to decline in

production and quality of eggs. Eggs are deformed and have watery egg yolk and soft or rough shells. Effective measures should be taken at this stage, otherwise, egg production would not come back to the normal levels resulting in economic losses (Cavanagh, 2007). In egg-laying birds, the abdominal cavity may contain yolk, which is also an indication of the reduced egg production (Ahmad *et al*., 2007). Although the alimentary form is not very common, enterotropic IBV does occur affecting the alimentary tract and may show signs of hemorrhages or ulceration in the organs like Proventriculus and Ceacal tonsils. and also cause thickness of the duodenum (Escorcia *et al*., 2002).

Clinical signs

Infectious bronchitis disease occurs in birds of all ages although young birds under 3 weeks are more prone to IB. Major signs of the disease are respiratory discomfort and a decrease in egg production with poor-quality eggs (Lee *et al*., 2004). Other clinical signs include cellulitis of periorbital tissues, edema, lacrimation, and frothy conjunctivitis. The affected birds become lethargic and reluctant to move (Terregino *et al*., 2008). Clinical signs may include sneezing, gasping, listlessness, tracheal rales, and nasal discharges. Other signs include the clustering of birds and weight loss (Cavanagh and Gleb, 2008).

Clinical signs of nephropathogenic form include depression, excessive water intake and wet droppings (Cavanagh, 2007).

Diagnosis

Conventional and modern methods have been used for the diagnosis of IBV. Type of method used usually depends upon nature of sample and also with subject to the availability of facilities and materials. It also depends upon whether the test is performed in the laboratory or the field (Bande *et al*., 2016). Some of the commonly used tests are discussed below.

Serology

Previously, different serological tests i.e. neutralization (VN) and hemagglutination inhibition (HAI) were extensively practiced for diagnosing and serotyping IBV strains. These tests were also used to determine flock safety after immunization (OIE, 2008). The newly developed ELISA test is more reliable, sensitive, and can be easily performed in the regional diagnostic centers. However, the newer serotypes do not show cross-reaction with the usually existing antisera which reduces the effectiveness of these serological tests (Cavanagh and Gelb, 2008).

Virus isolation and identification

Isolation of virus is the most important step for diagnosis of IBV. It is important to collect the samples at the onset of the outbreak. Recommended samples are trachea, Proventriculus, caeca, oviduct and kidney. Tracheal swabs are required to be kept in PBS or buffered solution before they are transported to the lab. All the samples must be obtained aseptically and placed in air-tight plastic bags (Bande *et al*., 2016).

Embryonated chicken egg

The allantoic cavity of 9-11 days old embryonated chicken egg is considered as one of the best route for the growth of IBV. Sample suspension is inoculated into the allantoic cavity of specific pathogen free embryonated chicken egg followed by incubation at 34-37°C. The inoculated eggs are candled daily to observe embryo viability. Embryos showing death within 24 hours are considered nonspecific. After 2-3 days of virus inoculation, allantoic fluid is collected from the egg and verified for the existence of IBV by using hemagglutination or RT-PCR technique (Bande *et al*., 2016). Sometimes it is necessary to pass the allantoic fluid through several blind passages so that virus can adapt to eggs and produce high titers. This increases the time needed for an accurate diagnosis. The eggs are examined for IB lesions i.e. twisting and dwarfing. It is notable that these are not the specific signs of IB but suggestive only (Bande *et al*., 2016).

Cell cultures

Various primary and secondary cells have been used for the isolation of IBV. These cells are chicken embryo kidney, chicken embryo fibroblast and Vero cells (Arshad and Al-Salihi 2002). Infected cell cultures show characteristic signs of rounding, syncytial development and succeeding detachment from the plate surface. The major drawback of using this method is that all strains of IBV do not adapt to cell cultures easily although some strains do adapt e.g., M41, Iowa 97, and NZ. Some strains require several passages in embryonated eggs before culturing in cell cultures. Sometimes cell cultures give very low viral titer or even fail to grow IBV (Bande *et al*., 2016).

Organ culture

Another method used for the detection of IBV is

the tracheal organ culture (TOC) method. Tracheal rings of 20 days old chick embryos are used for the preparation of TOC. This method applies to both embryo-adapted and non-adapted IBV strains. Tracheal rings of chicken embryos are kept in roller bottle and inoculated with apparent sample. Using light microscope, the culture is examined for the presence of ciliostasis. The culture is considered positive if the ciliary activity becomes impaired completely (Jones and Hennion, 2008). This method has shown positive results for samples from oviduct, kidney, Proventriculus and intestine. However, the results vary with IBV strain and with the amount of virus present in the sample. This method has the advantage of easy titration and serotyping of IBV (Armesto *et al*., 2011). On the other hand, some strains do not have the affinity to grow in tracheal cells and it is often difficult to differentiate ciliostasis due to other viruses like avian adenovirus and Newcastle disease virus (Cavanagh and Gelb, 2008).

Electron microscopy

Electron microscopy is used to detect and identify IBV on the basis of its morphological characteristics. Culture is examined under the electron microscope for the evidence of coronavirus-like pleomorphic structures with spike projections. This step is followed by negative staining with phosphotungstic acid. Notably, the shape and diameter (120 nm) of coronavirus is taken into consideration while detecting the virus.

Immunobiochemistry

There are two significant histo-chemistry procedures which are used for diagnosing and identifying IBV are immunofluorescence and immunoperoxidase. The basic principle of these methods is antigen-antibody reaction (Bezuidenhout *et al*., 2011). Immunofluorescence is the most widely used technique and is usually conducted on collected allantoic fluid (Abdel-Moneim *et al*., 2009) while avidin-biotin complex (ABC), which is developed in immunoperoxidase method, has been successfully used to find IBV in tissue samples (Abdel-Moneim *et al*., 2009).

Molecular diagnostic assays

Conventional serological techniques and virus cultivation methods used to detect IBV have now been replaced by molecular diagnostic assays e.g., RT-PCR, real-time RT-PCR, Restriction Fragment Length Polymorphism (RFLP), and genome sequencing because of their sensitivity and rapidity (Zhu *et al*., 2007).

RT-PCR methods

The principle approach of this method is using viral RNA. Amplification is done either by using one-step RT-PCR (directly) or two-step RT-PCR (following cDNA synthesis). This method was designed in 1991 for the detection of IBV-S1 gene. Later, other RT-PCR methods including general and serotype-specific assays were introduced to target different sections of the IBV genome (Keeler *et al*., 1998). Due to the conserved nature of the target section in various IBV serotypes, the UTR and N-gene-based RT-PCR are used universally for the detection of IBV. Pan-coronavirus primers targeting a conserved section of various coronavirus isolates is used in one-step RT-PCR amplification of IBV strains (Stephenson *et al*., 1999) but genomic sequencing and amplification of S1 gene is a reliable method for the classification of new strains of IBV (Zhu *et al*., 2007).

Restriction fragment length polymorphism (RFLP)

This method is used to identify new variants and is also used for the differentiation of various known strains of IBV. The entire sequence of S1 glycoprotein of IBV is subjected to amplification and enzymatic analysis (Mardani *et al*., 2006). The basic principle of RFLP is that it differentiates known strains of IBV on the basis of their specific electrophoresis banding pattern demarcated by restriction enzyme digestion. This method is similar to the conventional virus neutralization method but some strains like JMK and Gray could not be differentiated via this assay, which restricts the use of this method universally (Montassier *et al*., 2008).

Real time RT-PCR and other forms of PCR assays

This method not only provides more sensitive and specific results but also provides quantitative result of virus load present in the sample, which is based on the number of virus copies or fold changes (Callison *et al*., 2006). It also differentiates one IBV strain from other by targeting the S1 glycoprotein gene (Acevedo *et al*., 2013). Recently another method based on real-time PCR has been developed to differentiate field strains of IBV from vaccine strains and for the detection of recombinant variants. This method is known to have high-resolution melt curve analysis (HRM) (Hewson *et al*., 2009; Hewson *et al*., 2010).

Sequence and phylogenetic analysis

In this method, S1 gene is amplified by RT-PCR. After genotyping, it is subjected to bioinformatics analysis (Zulperi *et al*., 2009; Abro *et al*., 2012). On the basis of their phylogenetic resemblance with the sequences present in databases (EMBL, DDBJ and NCBI), isolates are characterized through bioinformatics analysis. It is notable that due to the lack of calibration among various laboratories, especially with respect to S1 sequencing, which is used in phylogenetic analysis, the use of this method is restricted. Recently, another molecular diagnostic assay i.e. Next Generation Sequencing (NGS) has become available, which provides the sequence of the whole genome in a short period of time (Bande *et al*., 2016).

Vaccination

The most effective tool to combat IB is vaccination. In many countries, low-virulence vaccination is being done in day-old chicks in hatcheries, which is followed by a booster dose of virulent vaccine in drinking water. The low virulence vaccine protects the birds from respiratory reactions, which may develop after highly virulent vaccination. There are two types of vaccines, which are formulated in oil emulsion adjuvants i.e. live attenuated and inactivated vaccines. The former is used mainly in preliminary vaccination of layers and breeders and may also be used in broilers (Ladman *et al*., 2002; Jackwood *et al*., 2009). The inactivated vaccine can only be beneficial if birds were formerly vaccinated with a live vaccine. Under conducive environments, immunity built due to vaccination may be effective for several months and even for a life time of the bird (Bijlenga *et al*., 2004).

Live vaccine

The Massachusetts strain H120 is the widely used strain in live vaccine. It is a mild vaccine and is used for first-time vaccination with short course of immunity. In areas where outbreaks of IB are high, this vaccine is generally used to protect the birds from respiratory problems. Vaccination can be done either via eye drops, intranasal route or by mass vaccination i.e. drinking water or spray. Vaccination with these techniques is economical and results in the development of both local and systemic immunity. The only drawback of using this vaccine is certain vaccination reactions, which may persist for some days (Matthijs *et al*., 2003; Bijlenga *et al*., 2004). The another Massachusetts strain i.e. Ma5 vaccine is a mild vaccine, which can be included in the preliminary vaccination program along with IB 4/91 vaccines for vast protection against various types of IB serotypes. Generally, breeder and layer flocks are vaccinated with live vaccines in order to protect them from indigenous respiratory tract problems. Immunization with live vaccines is highly recommended in areas where field cases are constantly diagnosed. It is notable that vaccine selection should be based on the prevalence of IB strain in a particular area/country. Some vaccine strains also develop cross-protection against the homologous and reference strains (De-Wit and van de Sande, 2009). Cross-protection against heterologous strains may also be developed by using a combination of Mass and Conn or Mass and JMK vaccines. There are many serotypes of IBV, which makes it difficult to control IB via vaccination. Therefore, only those vaccines are effective that are against the prevalent strains in an area/region. The most popular strain of IBV is the Massachusetts strain, M41 because it characterizes most of the isolates conveyed from several countries (Gelb *et al*., 2005; Terregino *et al*., 2008). For specific protection against IB, IB 4/91 variant having 793/B serotype or IB 274 vaccine virus containing D207 serotype are used. These vaccines are joint with Ma5 and IB multivalent vaccines for vast fortification against IBV (Mase *et al*., 2008).

Inactivated vaccine

Inactivated vaccine develops a long-term immunity and it also does not have any reactions. Inactivated vaccine is generally used at the time of laying in order to relieve stress and production loss (Terregino *et al*., 2008). These vaccines are costlier than live vaccines. Although high level of circulating antibodies is produced by the inactivated vaccines, the modified live vaccine still plays a vital role in shielding commercial layers by inducing better response to T cells and interpreting higher levels of local antibody i.e. IgA stimulation. In order to exploit the full potential of inactivated vaccines, the birds must be pre-vaccinated with a live vaccine. Thus, the highest antibody titers will be obtained after 4-6 weeks, vaccination period between last live and inactivated vaccine (Ladman *et al*., 2002).

Vaccine usage in various countries

In North America, vaccines for Arkansas, Massachusetts and Connecticut serotypes are available in both modified live and inactivated water-in-oil emulsion forms. In the USA, California strain and Georgia 98 vaccines are also used. Holland variants

(D-274, D-1466) are commonly used in Europe. In many parts of Europe, IB H120 vaccine is also being used. In Australia Vic S vaccine is widely used. K2 vaccine is believed to be useful in controlling emerging IBV recombinants (new cluster 1) and variants (new cluster 2) in Korea (Lim *et al*., 2012). The IBV vaccines used in developing countries are mostly imported from Europe, Canada, USA and Australia are unable to combat the local stains. Outbreaks despite of vaccination is therefore observed.

Future vaccines

A variety of advanced vaccines have been developed and tested experimentally. These vaccines are novel vaccines like DNA vaccines, sub-unit vaccines and vectored vaccine using S1 glycoprotein gene and reverse genetic vaccines (Dhama *et al*., 2008). Immunization against IBV has been transformed with the development of spike protein-based DNA vaccine (Sylvester *et al*., 2005). With this type of vaccine, not only the local strains of IBV can be controlled but also problems associated with live attenuated vaccines that sometimes become virulent can be surpassed. Other advanced vaccines, which are called recombinant vaccines or vector-based vaccines have also been developed. These are multivalent vaccines containing antigens of two or more viruses, which produce immunity against two or more diseases. Another revolution in this area is the DNA vaccine, which has shown satisfactory results in initial clinical trials. These vaccines need to be tested at large experimental scales before bringing into the market for commercial purposes (Yu *et al*., 2001).

Biosecurity measures for IBV control

Infectious bronchitis is a contagious disease, which spreads from bird to bird and from farm to farm. Measures that prevent the entry of the virus into the flock should be undertaken. These include strict biosecurity and good hygienic and sanitation practices accompanied by regular vaccination programs. All in all out method reduces the chances of disease outbreaks. Poultry farm should be stocked/restocked with disease free day old chicks. Proper sanitary measures should be adopted in cleaning and disinfecting the farm. Possible steps that can be taken to limit the introduction of virus in the farm and its intensity of infection include restriction of visitor access to the farm premises and movement of farm workers in and between farms. There should be separate clothing, footwear, and equipment for each farm. Footbaths with proper disinfectants should be kept at entry point of all farms. These steps are very important in order to prevent the virus from entering the farm. After the efflux of flock, all the organic material should be disposed of properly, away from the farm. The house should be thoroughly washed with 35-55 bar water pressure with the addition of detergents. IBV can be easily killed with disinfectants like formaldehyde, chlorine releasing, and quaternary ammonia compounds. Using these disinfectants with appropriate concentration and at regular intervals is very important for prevention and control. There must be a gap of 10-14 days between the two consecutive flocks in a farm (Welchman *et al*., 2002; Sylvester *et al*., 2005; De-wit *et al*., 2010; Dhama *et al*., 2011).

Conclusions and Recommendations

Infectious bronchitis is prevalent in poultry industry across the world. New stains are emerging in developed as well as developing world despite vaccination. Though mortality in growing flocks is not serious, the damage to the reproductive system in laying birds and breeder flocks is more alarming for the producers. The current literature search has revealed that measures so far adopted to control infectious bronchitis are not enough. The incidence of losses due to IB in the developing countries may aggravate in coming years due to their dependency on exotic vaccines. Further attention of the scientific community and funding agencies is needed to protect the global poultry industry from this challenge in the coming years.

Novelty Statement

This review article explains the different aspects of one the most important disease of the poultry with its worldwide distribution. It is an effort to attract the attention of world poultry experts for combating this economically important disease.

Author's Contribution

Sar Zamin Khan: Searched and reviewed the literature, arranged the findings and subtitles.
Muhammad Waqas: Wrote the manuscript.
Sagar M. Goyal: Reviewed the manuscript and corrected the language.

Conflict of interest

The authors have declared no conflict of interest.

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