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Emergence of Avian Infectious Bronchitis Virus and its Variants Need Better Diagnosis, Prevention and Control Strategies: A Global Perspective

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Abstract: Growth in poultry sector is being challenged due to increased incidence and re-emergence of diseases caused due to evolution of several viral pathogens and use of live vaccines. Piles of economic losses are encountered due to these diseases. Avian Infectious Bronchitis (IB), caused by *Corona virus*, is OIE-listed disease and characterized by respiratory, renal and urogenital involvements, causing high mortality. Economic losses are encountered due to loss of productive performance of both egg and meat-type chickens. Variant viruses evolve due to spontaneous mutations and recombinations, causing disease in vaccinated flocks of all ages. Serotyping and genotyping are the common methods of classification of IBV strains. The virus has 4 clusters, grouped into 7 serotypes and the most important strains are Massachusetts, Connecticut, Arkansas, Gray, Holte and Florida along with numerous others, distributed round the globe. Several conventional and molecular diagnostic methods have been described for the diagnosis of IB in chickens. 'All-in/all-out' operations of rearing along with good biosafety measures forms the basis of prevention, whereas vaccination forms the backbone of IB control programme. Both live and inactivated (oil emulsified) conventional vaccines are available. The new generation vaccines (recombinant and vector-based) developed against locally prevailing IBV strains may be more helpful and avoid the reversion of virulence in live vaccine viruses. The present review deals with all these perspectives of this important emerging poultry pathogen.

Key words: Infectious bronchitis virus, poultry, connecticut, massachusetts, nephritis, nephrosis, variant strains, diagnosis, prevention, control, vaccine

INTRODUCTION

The poultry farming in India was at its infancy during the 1960s which started as a small backyard venture. However, during the past three decades, the poultry farming has shown impressive performance powered by intensive system of rearing, scientific management, improved poultry breeds and ubiquitous use of vaccines. Poultry farming has developed into a vibrant, full-fledged organized commercial industry with the growth rate of 8-10% in egg production and 12-15 percent in meat production. As a result, India occupies 3rd, 4th and 6th rank in egg, layer and broiler production, respectively. However, presently the poultry sector faces challenges in several fronts. Increased incidence of disease outbreaks, re-emergence of various pathogens/diseases with higher virulence from time to time, evolution of variant strains due to recombination and simultaneous infection with multiple virus types and use of live vaccines are the reasons for such challenges. These diseases bring piles of economic loss in the form of inefficient feed conversion, decreased production, mortality, loss of market value, increased production cost etc (Morley and Thomson, 1984; Kataria *et al*., 2005; Rahul *et al*., 2005; Senthilkumar *et al*., 2003; Natesan *et al*., 2006; Bhatt *et al*., 2011; Dhama *et al*., 2008a, 2011a, 2013a; Singh *et al*., 2012; Gowthaman *et al*., 2012). One such common disease having all these features is avian Infectious Bronchitis (IB) (Hofstad, 1984; Ignjatovic and Sapats, 2000). The inclusion of this disease in the OIE (i.e., World Organization for Animal Health) list signifies its economic importance. It is a highly contagious and acute disease of

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chickens, caused by *Corona virus* (Cook, 2001; Lin *et al*., 2012). Avian Infectious Bronchitis Virus (IBV) is the causative agent. Infectious Bronchitis Virus is responsible for causing respiratory, renal and urogenital disease. Such disease is characterized by high mortality rates in affected flocks and severe economic losses due to production loss by reduction in weight gain by broilers and drop in egg qualityand production in laying poultry birds (Cavanagh, 2005, 2007; Almeida *et al*., 2012; Abro *et al*., 2012). Infectious bronchitis is prevalent worldwide, despite scientifically advanced poultry industry practices and availability of good vaccines and remains a global threat to poultry industry. It is possibly the most economically important viral respiratory disease of chickens in regions where there is no Highly Pathogenic *Avian influenza* Virus (HPAIV) or velogenic Newcastle disease virus. Antigenic shift and drift play important role in evolution of new strains. Infectious *Bronchitis virus* is known to affect multiple systems of the host involving lungs, oviduct and kidneys (Cavanagha and Gelb Jr., 2008; Cook *et al*., 2012; Jackwood, 2012). It causes respiratory problems, reduced performance, decreased hatchability and fertility, nephrosis and irreversible damage to reproductive organs (Cavanagh and Naqi, 2003; Jones, 2010). Vaccination is less successful because of continuous emergence of antigenic variance and less cross protection between the variance (Jackwood, 2012; Wang *et al*., 2012). Vaccination programmes should take into account the locally prevailing IBV strains to offer better protection against the field strains. Although the disease was first reported more than 80 years ago, control is yet to be complete (Cook, 2001).

The present review deals with various aspects of this important poultry virus having global significance and specifically describes the IB virus evolution and emerging variant strains, epidemiology and economic importance, the disease and pathogenesis. It also covers recent trends in diagnosis and vaccine development and appropriate prevention and control measures to be followed with an aim to combat IBV by designing/adapting effective disease control strategies. The review would be helpful for researchers and poultry producers for getting updated and comprehensive information about IB virus affecting the poultry industry.

METHODS

Infectious Bronchitis Virus (IBV), a Corona-like appearance-'Crown virus', is an enveloped, pleomorphic virus with a club-shape projection about 20 nm in length, approximately 120 nm in diameter, single stranded ribonucleic acid (ssRNA), 27.6 Kbp length genome with positive polarity (Jackwood, 2012). The virus is fairly labile and easily destroyed by disinfectants or organic solvents viz., ether and chloroform, sunlight, heat (56°C for 15 min), alkalis and other environmental factors (Van Roekel *et al*., 1941).

Classification and virus characteristics: Infectious Bronchitis Virus (IBV) belongs to order Nidovirales, family Coronaviridae, subfamily Coronavirinae, genus *Gammacoronavirus* and species *Avian corona virus* (ICTV, 2011). Among RNA viruses, the *Corona virus* has the largest genome, consisting of a 27.7 kb, single-stranded, positive-sense RNA (Kuo *et al*., 2013). IBV viral genome codes four major structural proteins: the Spike (S), Membrane (M), the internal nucleoprotein (N) and small membrane protein (E). Protective immunity, Hemagglutination-Inhibition (HI) and most of the Virus Neutralizing (VN) antibodies are induced by S1 protein that is formed by post translational cleavage of S protein. S1, the major antigen to which neutralizing antibodies develop, is the determinant of host species specificity and pathogenicity by determining susceptible cell range (tissue tropism) within a host. The N-terminal (S1) part of the S protein mediates attachment to cells via., Receptor Binding Domain (RBD) and is the most variable part of the S protein having unique amino acid sequences determining the serotype. Infectious *Bronchitis virus* serotypes (old and new) differ from one another, as well as from vaccine strains by approximately 50% of S1 amino acid sequence (Casais *et al*., 2003). The C-terminal S2 part triggers fusion of the virus envelope with host cell membranes. Important mechanisms of viral variation and diversity largely depend upon the large size of RNA genome, permitting diverse means of mutations and recombinations (Lai, 1996). At present, there is no definitive classification system. Serotypes are typed based on Virus Neutralization Test (VNT) after isolating them in embryonated eggs or cell culture system or in Tracheal Organ Cultures (TOCs), whereas protectotype of a strain is typed based on Cross-Immunization Study (CIS). Strains that induce protection against each other increase the efficacy of vaccination. Genotypes are grouped into strains based on genetic characterization using Reverse-Transcriptase-Polymerase Chain Reaction (RT-PCR), followed by Restriction Endonuclease (RE) cleavage site analysis and sequencing (Callison *et al*., 2006). Virus has the ability to mutate or change its genetic makeup very quickly. Numerous serotypes of IBV have emerged which complicate the control efforts through vaccination.

Replication strategies: The multifactorial regulation of translation during a IBV infection can be due to the N

protein, causing down-regulation of host cell translation in IBV infected cells (Hilton *et al*., 1986; Siddell *et al*., 1981), with upregulation of the translation of virus-encoded proteins (Tahara *et al*., 1994). The N protein may interfere with host cell translation by disrupting the formation of new ribosomes and possibly the cell cycle in the nucleolus, followed by binding to the 5' end of virus-derived RNA (Nelson *et al*., 2000). This causes recruitment of ribosomes for translation of viral RNAs. Alternatively, interaction of ribosomes with the *Corona virus* core can cause destabilization and release of genomic RNA (Hiscox and Ball, 1997), thereby causing trafficking of virus particles to the nucleolus in association with ribosomal proteins.

Evolution of IBV: The evolution of IB virus is constantly being reported due to intensive poultry farming practices, rapidly growing poultry industry, increased global trading and immense pressure of vaccines (Abro *et al*., 2012). New variants of IBV have emerged due to spontaneous mutation and recombination during virus replication, followed by replication of those phenotypes favored by selection (Abdel-Moneim *et al*., 2012; He *et al*., 2012; Toro *et al*., 2012a, b; Liu *et al*., 2013b; Mo *et al*., 2013) and cause significant disease in vaccinated flocks of all ages. It is assumed that due to widespread vaccination, the immune selection pressure involving the S1 subunit of S gene and the high mutation rate of the viral genome together might have resulted in the emergence of many serotypes and variants (Abro *et al*., 2012). Existing IB vaccines do not provide protection against these (Kuo *et al*., 2010). A new live-attenuated vaccine is required mainly based on locally prevalent field strains. Important IBV variants are: D274 and D1466 (Netherlands), B1648 (Belgium), AZ20/97 (Italy), Arkansas (USA), 84084 and 88121(France), 4/91(UK). Different environmental determinants within the host viz., immune responses, affinity for cell receptors, physical and biochemical conditions are also implicated in the selection process (Toro *et al*., 2012a). Recombinations in IBV S1 and N genes have been documented in several field isolates (Cavanagh, 2007; Kuo *et al*., 2010, 2013; Abdel-Moneim *et al*., 2012; Liu *et al*., 2013a). Variants may attain increased virulence, efficient receptor binding, rapid transmission and survival in host system. It is important to isolate and type the IBV virus strains prevailing in a geographical area time to time and vaccinate birds accordingly with vaccine strains that offer maximum cross protection against the field virus in question or use combination of different strains to provide broad protection. Similarly, vaccines can be developed from recently isolated IBV field strain(s) from a particular region. Amino acid substitutions in epitopes responsible for development of neutralizing antibodies can result in immune evasion (Shi *et al*., 2006). Thus, nucleotide sequencing and identification of amino acid substitutions especially involving S1 and N gene help to identify vaccine failure due to antigenic variation (Kuo *et al*., 2013). Variants are generally related to the appearance of positively selected, single point mutations or recombination in the antigenic domain of the viral proteins which occurs continuously (Lee and Jackwood, 2001; Jackwood *et al*., 2005; Kuo *et al*., 2013). These mutations lead to the alteration of virulence and the escape of the viruses from host defenses (Lee and Jackwood, 2001). The average synonymous mutation rate in all coronaviruses including IBV is approximately 1.2×10^{-3} substitutions/site/year (Hanada *et al*., 2004; Holmes, 2009). The use of modified live vaccines has helped in rapid evolutionary rate of IBVs (Jackwood *et al*., 2012). Understanding the evolution of IBV is essential with regard to development of control and prevention strategies.

Classification of strains: Serotyping by Virus Neutralization (VN) and Haemagglutination Inhibition (HI) and genotyping are the common methods of classification of IBV strains. Serotype specific monoclonal antibodies are induced by S protein (S1 subunit) but are available for a few serotypes in a small quantity. Owing to the emergence of novel variants it is difficult to have serum against all the strains. New serotype may emerge due to mutation. Nowadays, to classify (genotyping) strains, S1-specific RT-PCR is followed by sequencing or restriction endonuclease analysis (Abdel-Moneim *et al*., 2006; Sumi *et al*., 2012). Deduced S1 amino acid sequences with Virus Neutralization (VN) tests have revealed many serotypes. These serotypes differ by about 20-25% (amino acid), or by 40% or more occasionally, example being 7.6% difference between Conn 46 and Mass 41 strains in the S1 region or amino acid identity (97%) with D274.4 clusters, grouped into 7 serotypes based on RT-PCR analysis of the N gene and neutralization tests, respectively, were also identified (Wang *et al*., 1994; Roussan *et al*., 2009).

The Connecticut (Conn) isolate neither cross neutralize nor cross protect against Massachusetts (Mass) isolate (Jungherr *et al*., 1956). Florida, Clark 333 and Arkansas were other variants identified along with Holte and Gray (Winterfield and Hitchner, 1962; Brown *et al*., 1987; Butcher *et al*., 1989; Kinde *et al*., 1991). In North America, Massachusetts, Connecticut and Arkansas 99 IB viruses are common serotypes. A large number of variant viruses (82) have been reported,

however only GAV and GA98 were found to be implicated in widespread disease disseminations and persistent virus infections. Other IBV variants involved were Mass, Conn, Ark-DPI, CAV, DE072, MX97-8147, etc. (Jackwood *et al*., 2005). Three variants viz., CA557/03, CA706/03 and CA1737/04 were not related to each other or to Conn, Ark, or Mass vaccine strains, genetically or by cross-virus neutralization test (Ziegler *et al*., 2002; Kingham *et al*., 2000; Jackwood *et al*., 2007). Most of the Brazilian IBV field isolates recorded up to 1989 were classified as Mass (Massachusetts) serotype. Seroprevalence of the IBV in various species of birds in some of the countries (like Grenada) was reported, although vaccination against IBV is not practiced (Gutierrez-Ruiz, 2004; Sabarinath *et al*., 2011; Ramirez-Gonzalez *et al*., 2012). There are five distinct genotypes: A, B, C, D and Massachusetts based on the RFLP patterns of Twelve Brazilian isolates and one reference vaccine strain (De Fatima *et al*., 2008; Rimondi *et al*., 2009; Acevedo *et al*., 2012). Massachusetts-serotype vaccine is the only one type of live attenuated vaccine approved in Brazil, but the genotypes are greatly divergent and the most of them belongs to Non-Massachusetts types (Hipolito, 1957; Di Fabio *et al*., 2000; Villarreal *et al*., 2007, 2010; Brandao, 2010). Interestingly, in Argentina, putative genotypes have been observed (De Fatima *et al*., 2008; Rimondi *et al*., 2009; Acevedo *et al*., 2012). The Massachusetts type was first reported in 1948 in UK and subsequently in Holland new serotypes were reported, against which vaccines were developed (Kusters *et al*., 1987; Parsons *et al*., 1992; Cook *et al*., 1996).

In African continent, IB was observed from 1954 onwards (El Houadfi and Jones, 1985; Kelly *et al*., 1994; Thekisoe *et al*., 2003; Owoade *et al*., 2004, 2006; Ducatez *et al*., 2009; Mushi *et al*., 2006). Five field IBV genotypes with three new IBV genotypes different from vaccine strains Massachusetts type, the fourth genotype similar to vaccine strain MA5 (Massachusetts type) and the fifth genotype similar to vaccine strain 4/91 have been identified (El Bouqdaoui *et al*., 2005; Bourogaa *et al*., 2009, 2012; Susan *et al*., 2010). The Mass and H120 serotype vaccine isolates and D274 are the most common IBV strains found to be circulating in the Middle East (Roussan *et al*., 2009). Others variants reported were IBV variant 1 (IS/64714/96, IS/222/96, IS/251/96, 793/B) and variant 2 strains (IS/223/96, IS/572/98, IS/585/98, IS/589/98) (Callison *et al*., 2001; Meir *et al*., 2004; Mahmood *et al*., 2011; Ababneh *et al*., 2012). Mass, Ark, DE-072 and JMK serotypes of IBV have been documented, based on the results from the Hemagglutination Inhibition (HI) test (Gharaibeh, 2007) and D274 and 4/91 (793B) with the use of RT-PCR (Roussan *et al*., 2008). Variant viruses in Russia clustered into the six novel genotypes (Bochkov *et al*., 2006).

The Chinese isolates were separated into five genetic groups (genotypes) viz. (LX4, 4/91, JP, Gray and Mass) and seven serotypes based on S1 HVR I nucleotide sequences and virus-neutralization test, respectively (Chen *et al*., 2009; Li *et al*., 2012). Thirteen isolates belong to serotype I; two isolates, GX-NN10 and GX-YL2, vaccine strains H120 and Ma5 and one reference strain, M41, belong to serotype II; five isolates belong to serotype III; isolate GX-YL1 belongs to serotype IV; isolates GX-GL1 and GX-NN7 as well as one vaccine strain, 4/91, belonged to serotype V; isolates GX-YL8 and GX-YL9 belonged to serotype VI. Neutralization titers of isolate GX-NN12 were relatively low against all the antisera, so it belongs to serotype VII (Wang *et al*., 1997; Wu *et al*., 1998; Li and Yang, 2001; Yu *et al*., 2001a; Liu and Kong, 2004). In Thailand, analysis of S1 HVR I nucleotide sequences separated the viruses into two groups (I and II) (Pohuang *et al*., 2009). The sequence analysis of the S1 gene demonstrates that NIB Indian isolate PDRC/Pune/Ind/1/00 possesses a unique genotype compared to other reference strains of various countries and is unrelated to North American, European and Australian strains (Bayry *et al*., 2005). Recently, an Indian IBV strain (India/LKW/56/IVRI/08) revealed 99% homology with a Thailand strain (THA280252) and the other strain (India/NMK/72/IVRI/10) showed even greater homology with IBV strains from UK (4/91 pathogenic strain), Japan (JP/Wakayama/2/2004) and China (TA03) (Sunni *et al*., 2012).

The disease known as uraemia was first recognized in Australia in the late 1940s. Cumming (1963) isolated Nl/62 virus (synonym 'T') from one such case in 1962, a prototype of nephropathogenic strains of IBV (Cumming, 1963). They were found to differ antigenically from other IBV strains having no common epitopes on either the N or M proteins (Ignjatovic and McWaters, 1991). These findings have indicated that unusual changes are occurring in strains isolated in Australia. Infectious *Bronchitis virus* strains viz., Ql/88, Ql/89, N3/88, N6/88, Nl/89, N2/89, N2/90, N5/90, Nl/94, N6/94, V18/91, V19/91, V6/92, V9/92, Vl/93, V2/93 and V3/93, shared only minor antigenic similarity with the classical Infectious *Bronchitis virus* strains and clearly belonged to group 2 of novel strains. There is no clear-cut correlation between the S1 amino acid sequences and the nephropathogenicity of strains (Ignjatovic and McWaters, 1991; Sapats *et al*., 1996; Ignjatovic *et al*., 1997). In New Zealand, IBV was first isolated in 1967 and four serotypes (A, B, C and D) of the virus were described

in 1976 that were different from those present in other countries, based on virus neutralization tests. Subsequently, a vaccine was produced from one of those serotypes (A) which was said to protect against all serotypes and is currently used in layer and breeder flocks and are having similarity to Australian Vic S strain (Pohl, 1967; Lohr, 1976; McFarlane and Verma, 2008). Percent cross protection offered by commonlu employed vaccine strains against various known field strains has been described by Gelb *et al.* (1991). Infectious *Bronchitis virus* strains prevailing worldwide are listed in Table 1. Emergence of IBV strains and timeline of events in their evolution are depicted in Fig. 1.

Host range, age susceptibility and tissue tropisms: Host range of IBV has been enlarging. The domestic chicken has been widely regarded as the exclusive host for IBV. Infectious bronchitis virus is also reported in pheasants, racing pigeons, peacock, partridge and mallard and associated with respiratory disease, egg production and shell quality disturbance. Infectious bronchitis virus grows well in 9-12 days old embryonated chicken eggs via., intra-allantoic route, producing curling and dwarfing of embryo. Infectious bronchitis virus has been propagated in chicken embryo tissue (kidney, lungs, liver), embryonic turkey kidney, Vero cells and chicken tracheal organ culture (Meir *et al.*, 2004). Among all the age groups of birds the young chicks are most ill affected and resistance develops according to age. Female chicks may harbor certain genotypes of the virus in the oviductal epithelium due to lack of maternal antibodies and such hens are termed as 'false layers'; the phenomenon being recently observed w ith a new variant predominant in Europe. Age-related susceptibility to infections is suspected to be under the control of an immunological response which in chicks develops fully at 2-5 weeks of age. Serotype-Massachusetts and Connecticut show more affinity for respiratory tract, whereas, serotypes-Holte, Gray and Australian 'T' strain are associated with nephrosis (Capua *et al.*, 1999; Ignjatovic *et al.*, 2002). Under certain circumstances, IBV is thought to persist for a considerable time in infected birds at some privileged sites like kidney and caecal tonsils (Meulemans and van den Berg, 1998; Almeida *et al.*, 2012).

EPIDEMIOLOGY AND ECONOMIC IMPORTANCE

Infectious bronchitis is prevalent in intensive poultry production system, having a high incidence of infection

Table 1: List of the strains of IBV prevalent in different continents across the world

Continent	Prevalent strains
America	Mass, Conn, Florida, Clark333, Arkansas, GAV, GA98, CAV, MX97-8147, DE072, Gray, Holte, Iowa, JMK, CA557/03, CA706/03, CA1737/04, PA/Wolgemuth/98, PA/171/99, PA/1220/98
Latin America	Mass, Conn, Ark, 4/91(793 B), D274, SIN6, UADY, Cuba/La Habana/CB19/2009, Cuba/La Habana/CB6/2009, 50/96-Brazil, Chile 14, 22/97-Honduras
Europe	Mass, Italy 02, QX (D388), H120 793B (4/91 or Cr88), D207, D212, D3128, D3896, H52, H120, D387, V1385, V1397, D274, D212, D1466, D3128, D3896, D274, D1466
Africa	Mass, H120, IBADAN, TN20/00, TN200/01, TN335/01, TN295/07, TN296/07, TN556/07, TN557/07, Egypt/Beni-Seuf/01
Asia	Mass, H120, Ark, Gray, Ark99, CU-T2, Vic S, DE-072, JMK, D274, 793/B, IS/222/96, IS/251/96, IS/64714/96, IS/223/96, IS/572/98, IS/585/98, IS/589/98, IS/885/00, Egypt/Beni-Seuf/01, Sul/01/09, JP, SAIBK, LDT3, QX, KM91, K2, LX4, GX-G, GX-XD, LD3, LS2, LX4, LH2, LHI10, THA20151, THA40151, THA50151, THA60151, THA90151, PDRC/ Pnne/Ind/1/00, RF/04/98, RF/07/98, RF/08/98, RF/10/98, RF/11/98, RF/12/98, RF/16/98, RF/17/98, RF/21/98, RF/01/99, RF/02/99, RF/03/99, RF/04/99, RF/05/99, RF/06/99, RF/13/99, RF/15/99, RF/20/99, RF/02/00, RF/03/00, RF/04/00, RF/08/00, RF/12/01, RF/14/01, RF/02/02, RF/05/02, GX-NN09032, GX-YL5, DY07, CK/CH/SD09/005, TC07-2, Q1, YN
Australia	Nl/62, Vic S, Nl/88, Q3/88, Ql/88, Ql/89, N3/88, N6/88, Nl/89, N2/89, N2/90, N5/90, Nl/94, N6/94, V18/91, V19/91, V6/92, V9/92, Vl/93, V2/93, V3/93, A, B, C, D

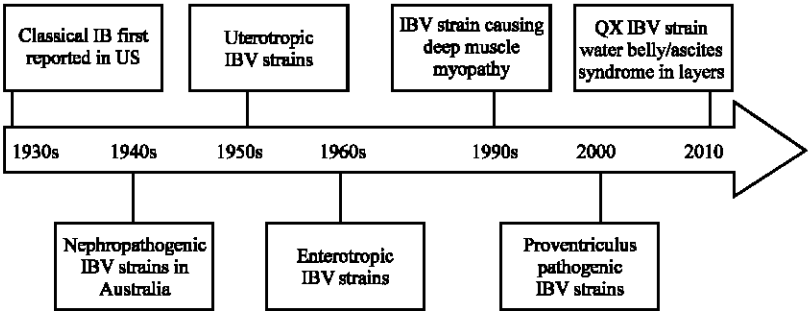

Fig. 1: Chronological enlistment of emerging IBV stains isolated from certain continents from different affected organs

with significant economic loss to the world poultry sector, despite proper vaccination. The virus has got different serotypes, prominent ones being Massachusetts type (M41), the prototype IBV, first isolated in 1941 and Connecticut (Conn strain) (Jungherr *et al*., 1956). Reports regarding IB have since been given from different parts of the globe in several poultry raising areas caused by unique strains except Australia and New Zealand. More than 30 serotypes and dozens of virulent variants have also evolved at times (Zanella *et al*., 2003). The incidence of nephrosis that is caused by variants of IBV is on the increase in many countries which necessitates the development of new vaccines (Winterfield and Albassam, 1984). Outbreaks of IB are often due to infections with the strains serologically different from the strains used as vaccines. Outbreaks mainly occur during winter (Lopez and McFarlane, 2006). The seasonal use of live virus vaccine (Ark) has led a subpopulation of the vaccine to revert back to virulence. Losses from production inefficiencies are more than mortality. In broilers IB leads to poor weight gain and loss of profit at slaughter. In layers, IB causes loss of egg qualities and egg production may drop down to 10-50%. Nephropathogenic strains cause mortality of up to 30% in susceptible flocks. In United Kingdom (U.K.), IBV is the biggest single cause of infectious disease-related economic loss (Pennycott, 2000; Meulemans *et al*., 2001).

Transmission and spread: Coughing and sneezing are mainly responsible for spread of the virus in chicken flocks. A short incubation period of 18-36 h is observed after IBV infection and thereafter clinical signs develop. Carrier birds rather than vectors are the nidus of infection. Disease can be transmitted through infected semen (Gallardo *et al*., 2011). There is no vertical transmission, ruling out the chances of infection related to management of hatcheries. Transmission from farm to farm is related to movement of contaminated people, equipment and vehicles. During an active outbreak air-borne spread of the virus over a considerable distance occurs. It is assumed that poultry industry in different parts of the world can contract the disease via migratory birds, even though the role of wild birds in the spread of IBV in most of the instances is speculative and largely unknown, deserving more attention and research activities (Sjaak de Wit *et al*., 2011).

Pathogenesis and the disease: Infectious *Bronchitis virus* has wide tissue tropism including respiratory, urogenital and digestive system (Boroomand *et al*., 2012) and has pathogenic effects in tissues of respiratory tract, kidney and oviduct. Infection occurs via respiratory route regardless of tissue tropism of strains. Virus multiplies chiefly in upper respiratory tract, following which viraemia occurs and the virus gets widely disseminated to other tissues. After the acute phase of infection, a persistent infection can be established in specific organs and the virus can be excreted continuously. The virus is primarily epitheliotropic and replicates in many epithelial cells, including those of respiratory tract, kidney and gonads producing lesions and in alimentary tract, many times with little pathobiological clinical effect (Ignjatovic *et al*., 2002). Infectious bronchitis virus infection of female chicks less than 2 weeks of age can cause permanent damage. Respiratory-urinary organs-digestive tracts-generative organ based transfer of the virus is responsible for tissue tropism (Benyeda *et al*., 2009). Infectious bronchitis occurs in birds of all age groups, but severely affects chicks evincing malaise, depression and retarded growth. Young birds of less than 3 weeks of age are more susceptible than older ones. The age and immune level of the poultry flocks and the pathogenic potential of the causative viral strain affects the nature and disease severity. Signs include respiratory symptoms, decreased egg production due to permanent damage to the oviduct and deterioration in egg qualities like watery albumin, misshapened and soft-shelled eggs. Recovery from the typical respiratory phase may occur in birds infected with nephropathogenic viruses which subsequently show signs of depression, ruffled feathers and wet droppings along with increased water intake (Lee *et al*., 2004). Mortality, as high as 25% or more occurs in chicken above 6 weeks of age group.

Respiratory tract shows serous, catarrhal, or caseous exudates in the nasal passages, sinuses and trachea. Caseous plugs may be found in the lower trachea or bronchi of young birds. Air sac affections are characterized by thickening and opacity. Focal areas of pneumonia may appear. In urogenital system, middle third of the oviduct is most severely affected and may be non patent and hypo-glandula, continuous patent but underdeveloped structure to a blind sac. Swollen and pale kidneys with the tubules and ureters often distended with urates. Despite lack of gross lesions in kidney, microscopic changes of nephritis may still be present (Abdel-Moneim *et al*., 2005). The abdominal cavity of chickens in production may contain yolk substances, a condition also observed in diseases leading to marked fall in egg production (Ahmed *et al*., 2007). Pathology is not usually associated with infection of the alimentary tract by enterotropic IBV. Pathogenic strains cause thickened, haemorrhagic or ulcerative lesion in the proventriculus, haemorrhagic lesions in the caecal tonsils and thickening of duodenum (Escorcia *et al*., 2002).

DIAGNOSIS

Infectious bronchitis can be diagnosed on the basis of clinical manifestation of disease, rising antibody titres and detection of antigen and/viral DNA in the tissue sections and clinical material. Infectious bronchitis virus can be isolated from the trachea, lungs and kidneys of infected chickens (Pradhan *et al*., 1982; Verma and Malik, 1971; Sylvester *et al*., 2003a; Dhama *et al*., 2011a, b) while liver and pancreas are candidates for antigen detection; trachea and spleen for histological diagnosis (Fan *et al*., 2012). Two or three blind passages may be required for primary virus isolation which can be a cumbersome and time taking process. Therefore, embryos of Specific Pathogen Free (SPF) chicken or their Tracheal Organ Cultures (TOCs) are preferred for isolation of IB virus (Cook *et al*., 1976). Further, direct detection methods in infected tissues include immunohistochemistry (Nakamura *et al*., 1991; Chen *et al*., 1996) or *in situ* hybridization (Collisson *et al*., 1990). Confocal microscopy can be used to detect the viral N protein both from healthy and uninfected cells and also reveals the presence of this important protein (required in viral replication cycle) in the host cell (Hiscox *et al*., 2001). Serological test include group specific Enzyme Linked Immune-Sorbent Assay (ELISA) (including monoclonal antibody based technique) (Lin *et al*., 2012), Virus Neutralization Test (VNT) (type specific) and Haemagglutination Inhibition (HI), Indirect Immunofluorescence Antibody Test (IFAT) and agar gel immunodiffusion (AGID). Fluorescent Antibody Technique (FAT) is also a good test for antigen detection. Nucleocapsid phosphoprotein gene-specific reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay has also been developed. However, for detection as well as serotyping, VNT is the gold standard test of choice while cross neutralization tests are used for detection of the variants (Elankumaran *et al*., 1999; De Wit, 2000, 2010; Chen *et al*., 2010). Tests involving detection of antigens should take into consideration that live IB vaccines are used ubiquitously and antibodies may fail to detect variant strains with modifies epitopes. Confirmation is made by nucleic acid-based methods: Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using-specific oligonucleotide primers, producing DNA copies of the S1 part of the spike glycoprotein gene to determine the identity of a field strain; Restriction Fragment Length Polymorphism (RFLP) RT-PCR and sequencing can identify all known serotypes of IBV as well as variant viruses (Meir *et al*., 1998, Sylvester *et al*., 2003b, 2006; Almeida *et al*., 2012; Jackwood *et al*., 2012; Sunni *et al*., 2012). Reverse Transcription-Polymerase Chain Reaction product is digested with a set of specific restriction endonucleases; the generated fragments are separated by gel electrophoresis and the specific pattern of their separation in the gel is compared with those of the standard strains for identification. Genotype identification is achieved by sequencing of the S protein (S1 subunit) gene. In US and Israel, RFLP analysis and RT-PCR product cycle sequencing are being used to identify filed strains (Zwaagstra *et al*., 1992). Recently, the improvements in the PCR technique have created way for the application of real time-PCR for very rapid detection and quantification of virus (Jackwood *et al*., 2003); multiplex-PCR and multiplex nested RT-PCR technique for differential diagnosis of IB infection (Kataria *et al*., 2005; Dhama and Mahendran, 2008; Chen and Wang, 2010; Nguyen *et al*., 2013). RNase T1 fingerprinting analysis can be used for fingerprinting of the IBV genome, advantage being use of complete genome for generation of strain-specific fingerprints (De Wit, 2000; Alvarado *et al*., 2005; Dhama *et al*., 2011b).

Infectious bronchitis virus lesions and PCR based diagnosis are presented in Fig. 2.

DIFFERENTIAL DIAGNOSIS

Infectious Bronchitis may resemble other acute respiratory diseases viz., Newcastle Disease (ND), infectious laryngotracheitis (ILT) and Infectious Coryza (IC) that occur more severely and causes greater production losses when compared to IB. Moreover, nervous symptoms of ND, slow nature of spread of ILT, or facial swelling of Infectious coryza are absent in case of IB. Production declines and shell quality problems in flocks infected with the Egg Drop Syndrome (EDS) are similar to those seen with IB, except that internal egg quality is not affected in case of EDS. Nutritional deficiency disorders must also be taken into consideration while diagnosing IBV (Sylvester *et al*., 2005; Cavanagha and Gelb Jr., 2008).

PREVENTION AND CONTROL

Biosecurity for prevention of the infectious bronchitis virus: Prevention and control measures mainly include follow up of strict biosecurity, good hygiene and sanitation practices, along with judicious vaccination programme. A one-age system ('all-in/all-out' operations) of rearing, cleaning and disinfection between batches will reduce the level of infection. Good management practices comprise of strict isolation/quarantine, restocking/repopulation with disease free day old chicks and adapting appropriate cleaning, disinfection and hygienic measures in the poultry farm. Steps to minimize

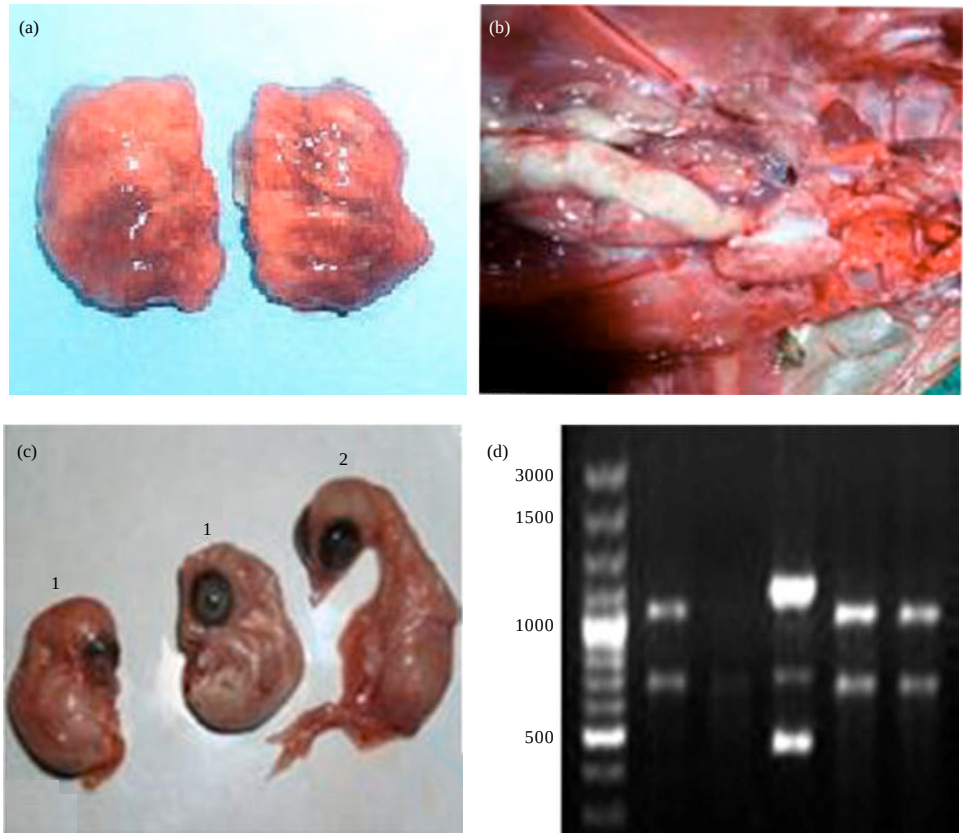

Fig. 2(a-d): IBV lesions and PCR based diagnosis (a) Lungs of experimentally infected chicks showing areas of consolidation, (b) Swollen ureter distended with urates in a chicken naturally infected with nephropathogenic IBV, (c) IBV infected chicken embryos (1) Exhibiting dwarfing and curling compared with uninfected embryo (2) and (d) RFLP pattern of PCR-amplified S1 gene (1720 bp) of vaccine (lane 1, 2) and field (lane 3, 4, 5) IB viruses

the intensity of infectious virus and limiting its introduction in poultry houses include controlled visitor's access of the farm premises; keeping separate clothing, footwear and equipments for each farm/unit; controlling movement of farm workers/personnel and equipments between farms and keeping appropriate footbaths with disinfectants at the entry points. These check points are very crucial for prevention and control of IBV on multi-age sites/farms and at times require a challenging job to be followed. All organic material should be removed or/and disposed from the house (top 4-5 cm of soil also removed from earth floor) and cleaning the houses at 35-55 Bar water pressure (adding detergents in the cleaning process is also advisable). Even though IBV is easily killed, use of suitable disinfectants (formaldehyde or chlorine releasing, quaternary ammonium compounds) with appropriate concentration and an optimum working time is very crucial to minimize infectivity of any residual virus particles. Disinfectants can be very helpful for preventing virus infections in a farm. Successive chicken flocks must be restocked with a minimum of 10-14 days downtime between them (Welchman *et al*., 2002; Sylvester *et al*., 2005; De Wit *et al*., 2010; Dhama *et al*., 2011a).

VACCINATION

Vaccination is the main key for the prevention and control of infectious bronchitis virus. In most of the countries, day-old chicks are being vaccinated in hatcheries itself with low virulence IBV vaccines. Thereafter, a booster immunization is followed with virulent vaccines, usually in the drinking water. This low virulence is suitable for chicks with a lower level of maternal immunity and they do not cause the respiratory reactions which can occur with vaccines of higher

virulence; disadvantage being the low level of immunity only enough to protect the respiratory tract (Kataria *et al*., 2005). Two types of vaccine viz., live attenuated and inactivated and killed are usually formulated in an oil emulsion adjuvant. Live vaccines are used in broilers and for the initial vaccination of breeders and layers while inactivated (oil emulsified) vaccines are administered in breeders and layers mainly during laying (Ladman *et al*., 2002; Jackwood *et al*., 2009). Nevertheless, for inactivated vaccines to be effective birds should have been previously "primed" with a live vaccine. Serial passages of IBV strains is followed in Embryonated Chicken Eggs (ECE) in order to achieve the required level of attenuation so as to be used in live vaccines. With optimal conditions, vaccination may give immunity for many months and this may be life-long (Cook, 2001; Bijlenga *et al*., 2004).

Live vaccines: These are Massachusetts strains: H120 vaccine (the most common representative of live vaccines) which is a mild vaccine and is known for the typical level of attenuation due to the number of passages it has undergone. It is usually used for vaccinations for the first time without inducing a long lasting immunity, in areas where there is increased level of field challenge with the intention of keeping the local protection of the respiratory tract at high level. Initial vaccination can be done individually by eye drop, intra tracheal or intranasal route or by mass vaccination (e.g., coarse spray or drinking water). Such procedure is usually inexpensive; induce local as well as systemic immunity. But unfortunately it can cause some vaccination reaction observed for a few days after vaccine application (Matthijs *et al*., 2003; Bijlenga *et al*., 2004). Ma5, a single component vaccine, is a mild one, used as a single component and can also be included in first vaccination programs with IB 4/91 vaccines and inactivated vaccines for broad protection against different IBV serotypes. Live vaccines are generally used in breeders and layers (young birds) to keep a good level of local protection of the respiratory tract and are advisable in areas of increased level of field challenge. However, selection of vaccine strain must be based on the strains prevalent in the area/country. Different vaccine strains in SPF chickens provides cross-protection against homologous and reference strains and variant field isolates (De Wit and van de Sande, 2009). Higher levels of cross-protection to some heterologous strains are given by combination of Mass and Conn or Mass and JMK. The occurrence of multiple serotypes of the virus has complicated and increased the cost of disease prevention and warrants the use of local strains in vaccines for their effective control. The inactivated vaccines are used primarily at the point of lay to avoid stress and loss of production. The Massachusetts (Mass or M41) strain is the most popular one as it is the representative of the initial isolates reported from many countries (Gelb *et al*., 1989, 1991, 2005; Cook *et al*., 1999; Terregino *et al*., 2008). In order to give specific protection against the IBV type, IB 4/91 variant virus (containing a strain of the 4/91 serotype) or IB 274 vaccine virus (containing a strain of the D207(D274) serotype) are used; when combined with Ma5 and IB multi vaccines, they provide broad protection (Mase *et al*., 2008).

Inactivated vaccines: Inactivated vaccines induce long lasting immunity, show no vaccination reactions, usually cost more than live vaccines and combination of different antigens apart from IBV can be achieved when given individually. Elevated levels of circulating antibodies were stimulated by inactivated vaccines than live vaccines, therefore it is useful in a breeder program where maternal antibody protection is needed. Still, due to induction of better T cell responses and rendering a higher local antibody (IgA) stimulation, modified live vaccines play significant role in protecting commercial birds (layers). Chickens must be properly primed with live vaccines, in order to exploit the potential of the inactivated vaccine and by this way, highest titres will be obtained at an interval of at least 4-6 weeks (period of vaccination between last live and inactivated vaccine) (Ladman *et al*., 2002). Further, vaccination programs may be simplified by combining inactivated antigens against two or more serotypes (or two or more diseases) into one vaccine (Hong *et al*., 2012).

IB vaccines employed in various countries: Both modified live vaccines and inactivated water-in-oil emulsions are available for the Massachusetts, Connecticut and Arkansas serotypes in North America; California strains and Georgia 98 vaccines being used in USA. In Europe, "Holland variants" commonly designated by number (e.g., D-274, D-1466) are documented; IB H120 based vaccine being used in most parts of Europe. IB Ma5 and IB 4/91 (as mentioned earlier) are live, freeze-dried vaccine serotypes, giving long lasting protection. Infectious Bronchitis D274 is a live vaccine against strain D274 in poultry for vaccination of future breeding and layers stock. Vic S vaccine is used in most vaccination programs in Australia. The K2 vaccine might be useful for the control of newly evolving IBV recombinants (new cluster 1) and variants (new cluster 2) in Korea (Lim *et al*., 2012).

Future vaccines: With advances in molecular biology, novel vaccines like DNA vaccines, sub-unit vaccine and

vectored vaccine using S1 glycoprotein gene, along with reverse genetics vaccines have been tried in different laboratories (Dhama *et al*., 2008b). Use of spike protein based DNA vaccine has revolutionized the concept of immunization against IB (Sylvester *et al*., 2005). Such tailor made-vaccines can be designed to suit the locally prevailing IBV strains and the problem of live attenuated strains reverting to virulence can be avoided. The recombinant or vector-based vaccines are also designed to introduce antigens from two or more viruses which act as multivalent vaccine giving protection against two or more diseases. DNA vaccine is another promising area as shown in initial clinical trials. These new generation vaccines can be administered safely in-ovo or to live chickens. The efficacy of these vaccines needs to be tested in large scale experiments before introduced for commercial purpose (Boots *et al*., 1992; Yu *et al*., 2001b).

It is the need of the hour to combat this economically important and emerging poultry pathogen by adapting and developing newer diagnostics, effective and safer vaccines, exploring novel therapeutics and following appropriate prevention and control strategies (Kataria *et al*., 2005; Sylvester *et al*., 2005; Cavanagh, 2007; Cavanagha and Gelb Jr., 2008; Dhama and Mahendran, 2008; Cook *et al*., 2012; Jones, 2010; Sjaak de Wit *et al*., 2011; Jackwood, 2012; Mahima *et al*., 2012; Deb *et al*., 2013; Dhama *et al*., 2008b, 2011a, b, c, 2013b, c, d, e, f, g, h; Tiwari *et al*., 2013).

CONCLUSION AND FUTURE PERSPECTIVES

Infectious bronchitis is an economically important disease of poultry having worldwide distribution, causing high morbidity (80%) and low mortality (20%). The economic losses are incurred upon by reduced egg production as well as worsen egg quality (internal and external); presence of silent layers; infertility and delay in growth; and increase in the susceptibility to secondary infections which altogether show the importance of the disease. Little or only partial cross-protection occurs between vaccine strains and new field strains which necessitate the development of new vaccines. The disease possesses a continuous threat to poultry despite of good vaccines, due to emergence of new IBV variants from time to time for which disease control by improved biosecurity, hygienic measures and vaccination programmes are required. For devising a strong IB control program, the presence of several serotypes, strains or variants of IBV and the possibilities of the emergence of novel ones have to be monitored. In this regard, detection and control strategies based on molecular biology and biotechnological tools and techniques are required to be developed. Alongside, upgradation of conventional techniques is also required to tackle the growing threat of IBV infection in order to effectively prevent and control it in the years to come. However, currently, better diagnostics and new generation vaccines are being developed along with the application of *in ovo* vaccination strategies so as to effectively check the spread of this ubiquitous and highly contagious viral infection of domestic birds.

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