

Review Article

Avian infectious bronchitis and its management in Nepal: a review

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Received: June 05, 2020; Revised: October 25, 2020;

Accepted: December 12, 2020; Available online: January 01, 2021

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ABSTRACT

Avian infectious bronchitis (IB) is a highly contagious disease of poultry with high economic importance. Caused by avian infectious bronchitis virus (IBV), it is transmitted by direct and indirect contact through aerosol or fecal means. Although IB is considered as respiratory disease, various strains of IBV affect the renal as well as the reproductive system. The economic importance of disease is due to lower egg production, poor hatchability of eggs, and decreased quality of the egg, weight loss, growth retardation, and high condemnation rates in meat-type birds. Although the prevalence of IB is lower in Nepal (>1%), it is ranked second as a disease which claims most livestock unit in the world. There is no specific treatment for IB but live and inactivated vaccines are available for the prevention and control of the virus. The lack of research in the infectious bronchitis virus can cause production losses in poultry sector due to the evolution of resistant virus strain in our country. This review discusses the aspects of avian infectious bronchitis prevalence in Nepal.

Keywords: Contagious, Infectious bronchitis virus, Poultry, Vaccination, Nepal

Correct citation: Rana, C., Bhattarai, B., Magar, K.B.R., & Panth, Y. (2021). Avian infectious bronchitis and its management in Nepal: a review. *Journal of Agriculture and Natural Resources*, 4(2), 211-226. DOI: <https://doi.org/10.3126/janr.v4i2.33773>

INTRODUCTION

Infectious bronchitis (IB) is a highly contagious acute respiratory disease of poultry caused by avian coronavirus, avian infectious bronchitis virus (IBV), and characterized by abnormal respiratory sounds, tracheal rales, coughing, and sneezing (Vegad, 2015). Chicken is the primary host but viruses also infect pheasants (*Phasianus colchicus*) and peafowl (Gough *et al.*, 1996; Felipe *et al.*, 2010). IB affects the respiratory tract, along with other non-respiratory tissues such as ovary, oviduct, and kidney of poultry depending upon the strain of the virus (Awad *et al.* 2014). The IBV is ubiquitous in distribution. IBV was first documented in the 1930s in the USA, since then more than 50 variants or serotypes have been reported which can persist from 12 days in spring to 56 days in winter (Cavanagh & Gelb, 2008). IB can cause respiratory disease i.e. acute respiratory tract infection, nasal discharge in young chicks, reproductive disease (loss in egg production, poor hatchability rate of fertile eggs, and

soft egg–shell with watery content in layers) (Almeida *et al.*, 2012), weight loss in both broilers and layers while nephritis in case of nephropathogenic virus (Awad *et al.*, 2014, Ganapathy, 2009). Study by Brandão *et al.* (2009) revealed the strong relationship between low immunity for the disease development. Morbidity of IB is up to 100%, whereas mortality is typically low about 20% to 30%, but in the case of nephropathogenic strain, mortality can rise from 5% to 90% (Ignjatovic *et al.*, 2002) along with secondary infectious agents such as *E. coli* or *Mycoplasma* leading to infections. The inefficient feed conversion, decreased egg production, mortality cause high economic loss (Kataria *et al.*, 2005; Senthilkumar *et al.*, 2003; Natesan *et al.*, 2006; Gowthaman *et al.*, 2012). However, the prevalence of IB in Nepal is very low which is around 1% (Gompo *et al.*, 2019). The objective of this review is to discuss about the situation of avian infectious bronchitis disease and its management in context of Nepal.

History of Avian Infectious Bronchitis

Infectious bronchitis disease was first observed in 1930 in the North Dakota, United States, and the first documented published in 1931 (Schalk & Hawn, 1931) as a consistent disease with infectious laryngotracheitis (ILT) and in 1936 IB and ILT were differentiated based on the causative agent (Beach & Schalm, 1936). IBV was first propagated in the allantoic cavity of embryonated eggs by Beaudette and Hudson in 1937 (Beaudette & Hudson, 1937). Discovery of immunizing value in IBV propagated in embryonated eggs (Delaplane & Stuart, 1941) led to the production of the first IB vaccination using the van Roekel M41 strain in 1942 (Roekel *et al.*, 1942). Since the 1950s, Egypt identified the IB variants which were isolated by neutralization test, shows a closely related to Dutch variant D3128 and Mass variant (Sheble *et al.*, 1986). Various IBV serotypes were first identified in 1956 (Jungherr *et al.*, 1956) and contributed to the understanding of the existence of specific virus serotypes and different serotypes did not provide cross-protection. In the 1960s, a report on cross interaction of IBV with Newcastle disease virus in embryonated eggs and cell culture led to combined IBV and NDV vaccines ever since (Raggi *et al.*, 1963). In the 1960s, two nephropathogenic strains Gray and Holte were isolated (Winterfield & Hitchner, 1962). In the early 1980s, and IBV was associated with swollen head syndrome causing several problems in southern Africa (Morley & Thomson, 1984). In 1990, Israel confirms IB Variant based on neutralization tests and molecular techniques (Meir *et al.*, 2004). Major progress in the diagnosis of IBV emerged in the 1990s with many laboratories defining the various new strains of IBV using molecular techniques. Nepalese scenario shows only few reported data in issues with IBV infection; however the clinical signs in poultry birds must not be undermined. IB has been statistically shown to be present in Nepal between 2007-2009 and 2014-2019 (OIE, 2020). Gompo *et al.*, (2019) reported IBV as a cause of 1% of poultry disease in Nepal. Only 0.21% were IB cases between July, 2014 to June, 2015 in Central Veterinary Laboratory, Kathmandu (CVL, 2015)

Global distribution

Avian infectious bronchitis has claimed the largest numbers of poultry life in the world along with Highly Pathogenic Avian Influenza (HPAI), echinococcosis. In the survey of 176 countries, IB is ranked as a disease with the second largest loss of poultry life after Highly Pathogenic Avian Influenza (HPAI) and is followed by Low Pathogenic Avian Influenza (LPAI), Newcastle Disease (ND), and Infectious Bursal Disease (IBD) (Figure 1) (OIE,

2011). With an outbreak in 20 nations, IB is ranked as the 29th non-zoonotic disease based on livestock unit loss. Following the first reported case of IBV in the 1930s, different variants of IBV have been reported worldwide as an ongoing problem or as a periodic outbreak. The virus has more than 30 serotypes and dozens of variants (Zanella *et al.*, 2003). The most prominent ones are Massachusetts type (M41), the prototype IBV and Connecticut (Conn strain) (Jungherr *et al.*, 1956). After the identification of the 'T' strain in 1962 in Australia, various IBV forms have been described besides, IBV has emerged separately from the rest of the world in Australia owing to its remoteness (Ignjatovic *et al.*, 2002). Latin America reported the first IBV isolate of Mass serotype in Brazil (de Wit *et al.*, 2010). A study done in Brazil shows that at least five different antigenic types were found in commercial chickens (Di Fabio *et al.*, 2000). In 2009, a report detected novel IBV variants in Nigeria and Niger which were antigenically and genetically distinct from other known IBVs (Ducatez *et al.*, 2009). Important strains of IBV, i.e. 793B or QX that have been found over Asia (Wang *et al.*, 1998), Europe (Worthington *et al.*, 2008), and Africa but hasn't found in the USA or Australia. While the major Arkansas strain is found only in the USA and pathogenic strain like D146 reported only in Western Europe. In Russia, the IB variant clustered into the six novel genotypes (Bochkov *et al.*, 2006). In Asia, recently many studies have been done in different countries. In 1967, IBV was first recognized in Malaysia which were a Mass serotype whereas Thailand reported IB in 1950, later studies in Thailand identifies two groups of IB variants by phylogenetic analysis of the S1 gene (Pohuang *et al.*, 2009). In 1998, China identifies a highly pathogenic IBV Variants associated with both respiratory disease and nephritis by in vivo studies and antigenic typing using monoclonal antibodies and cross-neutralization tests (Wu *et al.*, 1998). In China, a total of nine different genetic groups have been recognized: LX4, LDT3, LHLJ, BJ, LDL, N1/62, and LSC as well as Mass- and 793B-type viruses (Han *et al.*, 2011). Since 1991, the most common IBV types reported in India were the Mass and 793B types (Elankumaran *et al.*, 1999). It was clear that a large number of IB variants exist worldwide according to the geographical area however, their origin is not clear. In 2004, a study suggested that Asian variants are recombinant, derived from Australian variants and the N genes from US strains (Shieh *et al.*, 2004).

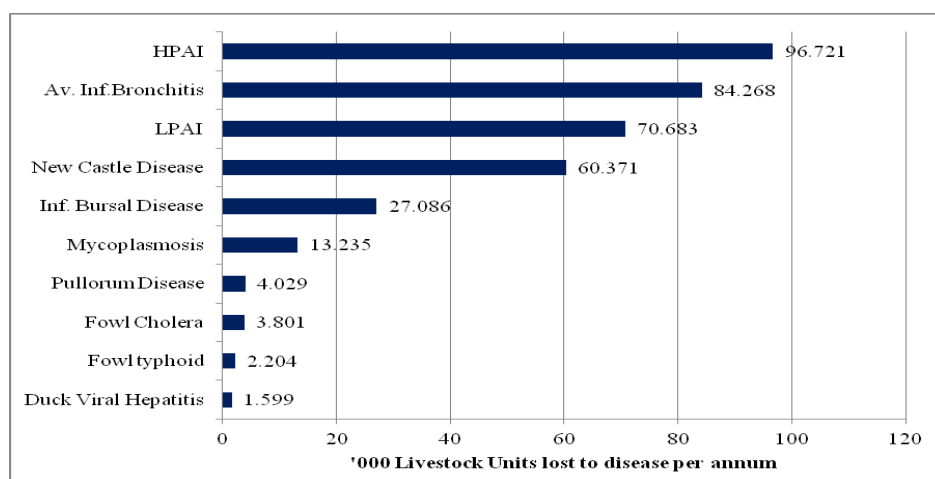


Figure 1: Poultry losses from poultry diseases from 2006-2009 (Source: World Livest. Dis. Atlas, 2011)

Note: LSU losses from all other poultry diseases: 2,760

Scenario of Nepal

Avian infectious bronchitis (IB) has been statistically shown to be present in Nepal between 2007-2009 and 2014-2019 (OIE, 2020) (Figure 2). IB is ranked among the top ten poultry diseases in Nepal alongside colibacillosis, myotoxicity, ascites, complicated Chronic Respiratory Disease (cCRD), Infectious Bursal Disease (IBD, Newcastle Disease (ND), Avian Influenza (AI)), Salmonellosis and Coccidiosis. Even though the disease was reported low prevalence (Gompo *et al.*, 2019), the economic loss due to negative effect in the productivity cannot be neglected. Seasonal occurrence and static regarding IB were not found in Nepal; however, it is reported that its prevalence was found all year round (Gompo *et al.*, 2019). Among the available cases, only 0.21% was IB cases were reported in between July 2014-Jun 2015 without specific season prevalence (CVL, 2015).

Causative agent

Infectious bronchitis in poultry is caused by group 3 coronavirus (Cavanagh, 2003), the avian infectious bronchitis virus of the Coronaviridae family. The Coronaviridae family contains two subfamilies, *i.e.* Coronavirinae and Torovirinae. Coronavirinae subfamily includes alphacoronavirus, betacoronavirus, delta coronavirus, and gammacoronavirus genera. Alphacoronaviruses and Betacoronaviruses are mammalian viruses while Gammacoronaviruses is an avian virus with avian coronaviruses IBV and Turkey coronavirus (TCoV) (Jackwood & Wit, 2020). The Coronavirus has the largest genome (27.7 kb), a positive-sense single-stranded RNA virus (Kuo *et al.*, 2013). This large size of the RNA genome permits the important mechanism of mutations and recombinations (Lai, 1996). Infectious bronchitis virus is a single-stranded enveloped RNA virus with a round to pleomorphic shape about 80-120 nm in diameter and contains four virus-specific proteins *i.e.* spikes protein (club-shaped surface projections) about 20 nm in length, membrane (M) protein, the nucleocapsid (N) protein and the envelope (E) protein (Thor *et al.*, 2011). Spikes protein gives the virus a crown-like appearance so the name corona (Figure 3). This spike glycoprotein is the most significant protein for virus identification because it contains epitopes for neutralizing and serotype-specific antibodies (Masters, 2006). Infectious bronchitis virus strains vary in their sucrose gradients density from 1.15 g/mL to 1.18g/mL (Davies & Macnaughton, 1979). The United States of America (USA) Animal Health Association Report (105) lists respiratory diseases as a major cause of economic loss to the broiler industry, among which IBV being the major causative agent of respiratory infection (Ramakrishnan & Kappala, 2019).

Transmission

Infectious bronchitis virus (IBV) has a short incubation period (IP) of about 18-36 hours. The IBV is highly contagious since it is transmitted by air through inhalation and ingestion of infectious virus particles and direct contact; and indirect transmission occurs through aerosol droplets or feces, exposure to fomites containing virus such as clothing, shoes, tools, etc. Aerosol transmission is a significant mode of transmission during the first 3–5 days PI due to high concentrations of virus in the respiratory tract (De Wit, 2000). In the respiratory tract, the viral load drops rapidly below the level of detection within the second-week post-infection (PI) (Wit, Jong *et al.*, 1998). The virus can also be transmitted through the feces and uric acid from the kidney. The virus can also be transmitted through infected semen (Gallardo *et al.*, 2011). In the chronic phase the occurrence of probable long-term excretion

and re- excretion through the colonic area may result in herd transmission by direct or indirect contact with contaminated litter, fomites, or personnel (De Wit *et al.*, 1998). Detection of viruses in the caecal tonsils (up to 14 weeks) and feces (up to 20 weeks) after infection suggest fecal transmission of infectious bronchitis virus (Alexander & Gough, 1977). Vertical transmission has not been yet reported but the viral shedding on the shell surface is evident (Jackwood & Wit, 2020).

I-VI: January -June; VII-XII: July-December



Disease absent



Disease present

2005		2006		2007		2008		2009		2010		2011			
I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII		
2012		2013		2014		2015		2016		2017		2018		2019	
I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII

Figure 2: Status of IB in Nepal between 2005 and 2019 A.D. (Source: OIE, 2020)

Chicken is the primary host but the virus is also reported in pheasants and peafowl (Gough *et al.*, 1996). Birds of all age and breed are susceptible but the severity is high in younger chicks with mortality between 15-90 %. Virus transmission occurs through various routes including inhalation and through direct contact, which upon entering the body replicates in the lungs and trachea in epithelial cells (Dhinakar & Jones, 1997). Then, Viremia occurs disseminating the virus to epithelial cells of various organs like the kidney, oviduct, testes, and Bursa of Fabricius depending on the infecting strain (Reddy *et al.*, 2016) causing damage to kidney tubules; reduced absorption of glucose, water, and electrolytes, which leads to dehydration and acidosis causing mortality. Mortality is high in the case of immunosuppression along with secondary infection.

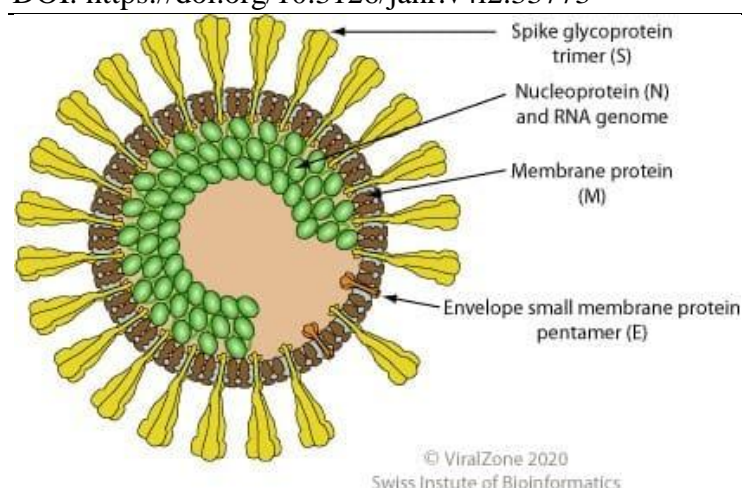


Figure 3: Morphology of Avian infectious bronchitis virus (Source: Aziz, 2020)

Clinical signs

The magnitude and clinical characteristics of IB infection depend on the virulence of the virus and organ or system involved. Chronic infection may last for several weeks, though clinical signs of uncomplicated infection last less than seven days with a mortality of 5% to 25%.

Table 1. Clinical signs of Avian infectious bronchitis

System Affected	Clinical signs	References
Respiratory	Panting, sneezing tracheal rales, lethargy, and nasal discharge from 1 to 4 days post-infection;Dyspnoea	Boroomand <i>et al.</i> , 2012; Terregino <i>et al.</i> , 2008
Ocular	Ophthalmitis, profuse lacrimation, edema, and periorbital cellulitis	McFerran <i>et al.</i> , 1972
Reproductive	A 3-10% reduction in egg production which may go up to 50%, along with misshapen egg and rough or soft-shelled egg with watery albumin; Decreased hatchability of eggs	Cavanagh, 2007
	Lower production returns to near normal typically within a week, although sometimes six to eight weeks can elapse; Birds may fail to come into production (false layers) when virus replication occurs in the oviduct because of permanent damage in young hens' oviduct	Swayne <i>et al.</i> , 2008; Benyeda <i>et al.</i> , 2009
	Infection in chicks less than two weeks of age can result in permanent damage to the oviduct, which leads to poor laying capacity	Jones & Jordan, 1972
Urinary	Hunched stance, reluctance to move, emaciation, excessive water intake; Wet litter; Death occur 4-5 days after infection	Cumming, 1969; Swayne <i>et al.</i> , 2008
Digestive	Proventriculitis; Enteritis; diarrhea, soiled vent	Yu <i>et al.</i> , 2001; De Wit <i>et al.</i> , 2011; Jones, 2010
General	Listlessness with ruffled feathers, reduced feed consumption, decreased feed conversion rate leading to weight loss and huddling near the source of heat	Cavanagh & Gelb, 2008
	General lethargy and disinclined movement	Terregino <i>et al.</i> , 2008

Diagnosis

Diagnosis of IB can be done by study of the clinical signs, gross and microscopic lesions, seroconversion, antigen and RNA detection depending on the study, the quality of research materials and equipment, the tracking period of the research, the aim of the test, and whether the test is performed in the field or the lab. Different diagnoses can be achieved by several techniques includes Serological test involving the Virus neutralization test (VNT) and hemagglutination inhibition (HI) test for detection and serotyping of Infectious Bronchitis Virus. VNT is considered as the gold standard test whereas cross-neutralization tests are used for variant detection (Chen *et al.*, 2010). IgM IgG-specific ELISA is mostly used in the field and monitoring antibody response as a more sensitive and easy technique (Martins *et al.*, 1991).

For virus isolation and identification, the fresh tracheal swab should be collected aseptically from slaughtered chicken and transported to the laboratory for further processing (Gelb *et al.*, 1998). Samples are inoculated into the 9-11-day-old chicken embryo's allantoic cavity and incubated at 34°C–37°C. Allantoic fluid (AF) is collected after 48–72 hours and checked for the existence of IBV via a serological or RT-PCR test. After 5–7 days, inoculated eggs are checked for characteristic lesions. Primary and secondary cells i.e. chicken embryo kidney (CEK) cells and Vero cells are also used for isolation and identification of IBV (Arshad, 1993). Limitation includes a possible lack of affinity of some strains to certain organ cells and distinguishing loss of cilia due to other respiratory viruses (Cavanagh & Gelb, 2008).

Morphology of IBV can be directly detected and identified using electron microscopy which helps in imaging virus-like particles in the infected sample cell (Patterson & Bingham, 1976). It is used for the study of the virus cycle rather than a specific diagnostic test (Arshad *et al.*, 2002). IBV antigen from sample tissue can be diagnosed by using Immunohistochemistry techniques using antigen-antibody reactions (Bezuidenhout *et al.*, 2011). The avidin-biotin complex (ABC) (Abdel-Moneim *et al.*, 2009) and indirect immunofluorescent assay (Yagyu & Ohta, 1990) are widely used immunohistochemistry tools for IBV diagnosis. The extensively used molecular diagnostic assay for IBV prognosis are Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), Restriction Fragment Length Polymorphism (RFLP), real-time PCR, and genome sequencing (Adzharet.*al.*, 1996). RT-PCR technique makes use of either direct (one-step RT-PCR) or cDNA (two-step RT-PCR) amplified viral RNA. A serotype-specific RT-PCR assay can be used for the differentiation of various IBV strains (Keeler *et al.*, 1998). Amplification and sequencing of the S1 gene are best to measure for genotyping of newly discovered IBV strains (Zhu *et al.*, 2007).

Unique electrophoresis banding patterns defined by restriction enzyme digestion are the base for the distinction of identified IBV strains (Montassier *et al.*, 2008). Real-Time RT-PCR Assays are more sensitive and specific (Acevedo *et al.*, 2013) thus, can be used for qualitative and quantitative analysis of IBV virus in sample tissue (Callison *et al.*, 2006). Differential diagnosis should be done with Newcastle disease (ND), Infectious laryngotracheitis (ILT), Infectious Coryza (IC), and Egg Drop Syndrome (EDS). While

making a diagnosis of IBV, nutritional deficiency disorder must be taken into consideration (Sylvester *et al.*, 2005).

Treatments

Infectious bronchitis has no specific treatment. But in case of mixed infections, antibiotics can be given against secondary bacterial pathogens. In the case of nephropathogenic IB, 72 mEq of sodium and/or potassium, with one-third in the citrate or bicarbonate salt form can be administered in the drinking water (Cumming, 1969). An additional heat source to prevent cold stress, good ventilation, avoiding overcrowding, and controlled feed consumption can be done to reduce losses from IB.

Prevention and control

A study done by Sharma and Tripathi (2015) showed an increased outbreak of poultry diseases in Chitwan is seasonal dependent, with spring season showing a higher number of disease outbreaks than winter, this emphasis on the strict application of the management and prevention strategy. Control and management procedures are difficult to prevent IBV infection due to the presence of a large number of IBV strains, with new strains evolving frequently. However, biosecurity and management improvements including strict isolation, repopulation of single day chickens upon cleaning and disinfection of the room, different age groups birds raised separately, and vaccination program can be done for checking the spread of IBV. Restocking after successive chicken flock must be with a minimum of 10-14 days intervals (Welchman *et al.*, 2002; Dhama *et al.*, 2011). Vaccination program through eye drops, nasal illustration, or aerosol sprays, drinking water is practiced. With wide variability, it is challenging to monitor infections despite commercially accessible live and inactivated vaccines. With the vaccine accessible, the correct approach for the management of IBV is to use vaccine strains that are close to those present in a specific farm or field. Activated or live vaccinations are commonly used in broilers as well as for the initial injection and priming of breeders and layer pullets.

Infectious bronchitis virus strains used in live vaccinations are attenuated by serial transit in embryonic chicken embryos (Bijlenga *et al.*, 2004), or combination with heat treatment (Jackwood *et al.*, 2010). Live vaccines are preferably administered intraocularly or intranasally but can be sprayed or put in drinking water (Cavanagh & Gelb, 2008). In breeders and layers, inactivated, oil-emulsion vaccines are injected intramuscularly or subcutaneously to induce high humoral antibodies levels. It provides protection against invading nephropathic and reproductive viruses in internal tissues mainly the kidney, reproductive tract to prevent production loss (Lee *et al.*, 2003). Maternal antibodies are critical to providing some defense against virulent IBVs (De Wit *et al.*, 2011). Continuous systemic surveillance of chicken flocks, as well as effective diagnosis, is required to achieve an appropriate epidemiological situation (Savic, 2017). Geographical isolation and monitoring mechanisms implemented such as restriction on cross-country trade in countries have an important role in preventing the spread of new IBV variants (De Wit *et al.*, 2011).

CONCLUSION

IB is a serious problem in a developing country where the poultry sector is just blooming. Incomplete removal of the virus from the source farm, inappropriate storage or

transportation of vaccines, poor farm management practices, etc. may be attributed to the risk of IBV infection in the Nepalese poultry scenario. The undermentioned prevalence may be due to inadequate studies on the disease. Although a low mortality rate, high morbidity rate, and high production loss account for a serious investigation of the disease. In our country due to low management practice, the contagious disease can be very challenging for chicks because of nephropathogenic strain. In addition, birds in Nepal suffer from different kinds of management stress, thus chances of occurrence of this virus and its effect on respiratory and reproductive performance cannot be neglected. Poor management practices including high flock density and contaminated litter play major roles in virus transmission between birds on the farm. Besides, the lack of research in the infectious bronchitis virus can result in large losses due to the evolution of resistant virus strain in our country. Illegal trading of poultry, as well as equipment between Nepal and India due to open borders, may result in the spread of virus between countries. Proper knowledge about the disease, strict government policy, proper management practice, and regular vaccination are essential measures to control and prevent disease.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. Subash Rimal and Mr. Jiban Shrestha for their unreserved support.

Authors' Contributions

C. Rana, B. Bhattarai, K.B. Rana Magar and Y. Panth wrote this review article.

Conflict of interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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