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Viral Diseases of Poultry in Assam, India: A Review

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ABSTRACT

The Indian poultry market is estimated to have an annual growth rate of 8.1% as of today. However, infectious diseases in poultry pose an important constraint in the growth and development of this sector in our region. Among infectious diseases, viral diseases of poultry pose a serious threat to the poultry industry from an economic point of view. Several viral disease outbreaks have been reported by various researchers from different parts of the country. Among the common viral diseases of poultry, incidences of Newcastle disease, Avian Influenza, Fowl Pox, Infectious Bursal Disease, Marek's disease, Infectious Bronchitis, Infectious Laryngotracheitis and Inclusion Body Hepatitis are significant in Assam as well as other parts of India. Thorough epidemiological studies followed by the identification of different serotypes, pathotypes, strains, etc. by genotyping and molecular characterization of viral disease pathogens may lead to ways to control and eradicate the diseases. Importance should be given to maintaining basic preventive measures like biosecurity, farm hygiene, and proper vaccination. In a developing country like India, disease outbreaks can impact the country's economy. In this study, a brief view of the common viral disease of poultry and its diagnosis and control strategies in Assam, India is depicted. However, this review well indicates a plethora of avian diseases that have occurred over the years causing a severe impact on poultry farming as a whole.

KEYWORDS: Diagnosis, economic effect, outbreak, poultry, viral diseases

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1. INTRODUCTION

The poultry industry in India has shown a remarkable growth rate of 8.51 and 7.52% in egg and broiler production, respectively (Anonymous, 2019). Estimates from the All-India Poultry Breeders Association indicate that poultry contributes to USD 17.31 billion of total India's gross value and satisfies the hunger of 50 million people through direct and indirect employment. The broiler and layer segments within the poultry sector constitutes about 65.3 and 34.7% of the total with a monthly turnover of 400 million chicks and 8,400 million eggs, respectively (Anonymous, 2020). However, several viral disease pathogens causes serious economic losses in India's commercial and backyard poultry farms almost every year. Among the viral diseases of poultry, incidences of Newcastle disease, Avian Influenza, Fowl Pox, Infectious Bursal Disease, Marek's disease, Infectious Bronchitis, Infectious Laryngotracheitis and Inclusion Body Hepatitis are significant in Assam as well as other parts of India. These diseases are considered to cause huge economic losses in the country. In brief, Newcastle disease (ND) is endemic in India and is a major constraint to poultry production and causes huge economic losses due to frequent outbreaks reported in both vaccinated and unvaccinated flocks (Gogoi et al., 2015, Kumar and Kumar, 2015, Morla et al., 2016, Das et al., 2021, Deka et al., 2022). ND has also been reported in wild birds (Gaurav et al., 2022). Similarly, avian influenza (HPAI) has caused billions of bird deaths with substantial impacts on poultry industries as well as hundreds of human deaths (Anonymous, 2019). Fowlpox generally occurs in backward poultry and has been reported in Assam (Pathak et al., 2017) causing economic losses to poor farmers. Infectious bursal disease results in significant economic losses worldwide (Li et al., 2013, Vukea et al., 2014) with new strains of IBDV regularly reported (Nandhakumar et al., 2020, Aliyu et al., 2021, Lian et al., 2022). Marek's disease causes annual economic losses in the poultry industry (Bertzbach et al., 2020). In India, periodical outbreaks of MD were reported in Gujarat (Kalyani et al., 2010), Tamil Nadu, and Karnataka (Raja et al., 2009, Muniyellappa et al., 2013), Uttar Pradesh (Kumar et al., 2018) and, recently, from North-east India (Puro et al., 2018) and Andhra Pradesh (Prathibha et al., 2018). Infectious Bronchitis Virus (IBV) poses a major global economic threat (Cavanagh, 2007, Laconi et al., 2020). In India, descriptions of IBV have been reported by Bayry et al. (2005), Sumi et al. (2012), Patel et al. (2015), Parveen et al. (2017) and Ganapathy et al. (2020). Infectious Laryngotracheitis outbreaks were reported in India by Srinivasan et al. (2012), Gowthaman et al. (2014), Sivaseelan et al. (2014), Baksi et al. (2016) and Gowthaman et al. (2020). In Assam, the outbreak of Inclusion Body Hepatitis has been reported in poultry from a few districts of Assam (Dutta et al., 2021).

Assam is the most prominent state in the North Eastern (NE) region of India with a large population of livestock and poultry. The majority of the population in Assam is non-vegetarian thereby having a very high demand for eggs and poultry meat in this region. The district-wise distribution of the poultry population in Assam as per the 20th livestock and poultry census is shown in Figure 1. Based on the above fact, the critical viral diseases that have been reported in poultry in Assam having economic importance are reviewed in this article.

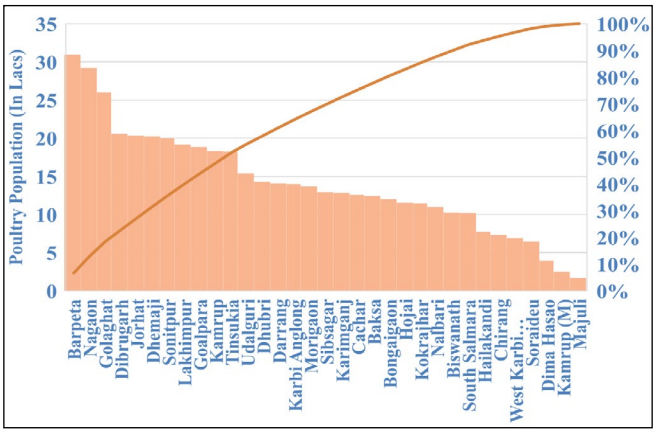

Figure 1: Pareto chart plot showing district-wise poultry population of Assam 2019 as per the 20th livestock census of Assam with secondary axis as a percentage of the total

2. NEWCASTLE DISEASE

Newcastle Disease (ND), also known as Ranikhet Disease, is regarded as one of the major fatal diseases of poultry as it causes considerable economic loss in terms of high morbidity, mortality, and a drop in egg production. The disease is caused by *Avian orthoavulavirus 1* (AOAV-1, ND virus (NDV) within the subfamily *Avulavirinae* of the family *Paramyxoviridae* (Anonymous, 2019). Pathotypes of NDV based on virulence and tissue tropism include Viscerotropic Velogenic isolates (Doyle's form), Neurotropic Velogenic isolates (Beach's form), Mesogenic isolates (Beaudette's form), Lentogenic isolates (Hitchner's form), and Asymptomatic enteric isolates (Alexander, 2000). The disease is worldwide in distribution. ND was first reported in Java, Indonesia in 1926 (Kranevald, 1926) and subsequently from different parts of the world (Doyle et al., 1927). It was first reported from India between 1928 and 1930 in Ranikhet (Edwards, 1928) and Madras- Chennai. Since then, it has been endemic in India, and regular outbreaks have been reported from different parts of India from commercial and backyard poultry (Gogoi et al., 2015, Jakhesara et al., 2018, Kumar and Kumar, 2015) as well as in wild birds (Gaurav et al., 2022). A study during the period 2013-2014 in India reported total economic losses of 37,19,223 rupees due to the ND outbreak (Khorajiya et al.,

2017). Nath et al., 2016, Nath and Kumar, 2017 reported different ND outbreaks in chicken flocks in Assam caused by virulent genotype XIII NDV strain during 2014-15. Virulent NDV was detected from the outbreak in broilers in Assam (Das et al., 2021). Deka et al., 2022 also reported an ND outbreak in the backyard and commercial poultry in Assam caused by genotype XIII NDV. Gaurav et al., 2022 isolated genotype VII NDV from barn owl in Guwahati, Assam.

A presumptive diagnosis is made based on clinical signs and lesions. Laboratory confirmation can be done by isolating and identifying the virus, which is considered a "gold standard" test (Anonymous, 2021). Virus isolation is carried out in 9-11th day-old embryonated SPF eggs. Serological diagnosis includes Haemagglutination inhibition tests, ELISA, and viral neutralization tests. Molecular diagnosis can be made by techniques like Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), Real-Time- Polymerase Chain Reaction (qPCR), etc., which are considered more sensitive than conventional assays (Anonymous, 2021).

3. AVIAN INFLUENZA

Avian influenza (AI) is a contagious viral disease of poultry characterized by high morbidity and mortality, respiratory signs, depression, reduced feed, and water intake. In egg-laying birds, there is a decline in egg production. It is a disease of poultry that is caused by infection with type A influenza viruses of the Orthomyxoviridae family (Swayne and Spackman, 2013). These viruses have a segmented, single-stranded, negative-sense RNA genome within the envelope (Neumann et al., 2009). There are many strains of AI viruses, and generally, they can be classified into low pathogenic (LPAI) and highly pathogenic (HPAI). All naturally occurring HPAIVs have been H5 or H7 subtypes, while LPAIV has been any of the H1–18 subtypes. Globally, disease from AIV, especially HPAI, has caused billions of bird deaths with substantial impacts on poultry industries as well as hundreds of human deaths (Anonymous, 2019). The avian influenza outbreak was first noticed in Italy in 1878 and is spreading globally. India was free from HPAI till 17th February, 2006. India experienced the first Highly Pathogenic Avian Influenza (H5N1) outbreak in Maharashtra and Gujarat on 18th February, 2006, followed by the second outbreak in Madhya Pradesh in March, 2006. Subsequent outbreaks were then reported from a small poultry farm at Chingmeirong in East Imphal district of Manipur during July, 2007; in Birbhum and Dakshin Dinajpur districts of West Bengal on 15.01.2008 and spread to other 13 districts of the state; in Salema Block of Dhalai district of Tripura on 7th April, 2008; on 27.11.2008 in Kamrup district of Assam and subsequently, the disease spread to eight more districts of the State i.e. Kamrup (Metro), Barpeta, Nalbari, Chirang, Dibrugarh, Bongaigaon, Nagaon and Baksa; in English Bazar Block of Malda District of West Bengal on 15th December, 2008; in Ravongla municipality in South Sikkim district in Sikkim, 2009; on 14.01.2010 in Khargram block of Murshidabad district of West Bengal; 2 outbreaks of Avian Influenza one each in Government Duck Farm R.K. Nagar, Agartala on 17.2.2011 and Government Poultry Farm Gandhinagar, Agartala on 6.3.2011 were notified; in village Bhamondanga Part-1 in Agomoni block in district Dhubri in Assam on 8th September, 2011; Nadia district in West Bengal on 19th September, 2011 and consequent reports from different states with latest report of outbreak from Panchkula District of Haryana during January and February 2021.

Diagnosis can be made by isolating and identifying the virus by inoculating clinical samples (respiratory organs) into the allantoic cavity of 9–11day old chick embryos (Anonymous, 2021). Serological tests like Agar Gel Precipitation Test, Complement Fixation Test, Fluorescent Antibody Technique, and ELISA can be employed. Molecular techniques like PCR and reverse transcriptase PCR (RT-PCR) can be done on oro-pharyngeal swabs, giving rapid results within 3 hours (Anonymous, 2021).

4. AVIAN POX OR FOWL POX

Avian pox is a common viral disease in chickens, turkeys, pets, and wild birds (Tripathy and Reed, 2013). *Avian poxviruses* belong to the genus *Avipoxvirus* of the Family *Poxviridae* within the subfamily *Chordopoxvirinae* (Thiel et al., 2005, Anonymous, 2018), having relatively sizeable double-stranded DNA (dsDNA) that replicate in the cytoplasm of infected cells. *Avipox viruses* affect more than 329 avian species across 76 families and 20 wild and domestic bird species orders globally (Bolte et al., 1999, Carulei et al., 2017, van Riper and Forrester, 2007). The most common form of this disease is characterized by proliferative wart-like lesions that are commonly restricted to the eyes, beak, or unfeathered skin of the body called as cutaneous form or dry pox, and the other form of infection is the 'wet' or 'diphtheritic' form which is characterized by lesions on the mucous membranes of the upper alimentary and respiratory tracts (Niemeyer et al., 2013, Tripathy et al., 2000, van Riper and Forrester, 2007). The geographic distribution of avian poxviruses is worldwide (van Riper et al., 2007). Most of the infections in backyard birds are reported from Asia. In India's (West Bengal) study, the fowl pox virus was detected in a backyard flock with pock lesions in the comb, eyelid, beak, and wattle. Sequence analysis revealed the presence of nearly full-length reticuloendotheliosis provirus within the genome of the fowlpox virus (Biswas et al., 2011a, 2011b).

The lesions are relatively diagnostic and can be readily confirmed by histological observation or electron microscopy

(Anonymous, 2018). Avipoxvirus can be grown on the chorioallantoic membrane (CAM) of embryonated chicken eggs, causing its thickening and typical focal and diffuse lesions, and is one of the identification tools of choice (Cunningham, 1973). Molecular diagnosis using Polymerase Chain Reaction (PCR) has proven to be the most sensitive technique for diagnosis of APV infection, which is mainly based on the amplification of a 578 bp P4b gene of APVs and is been increasingly used during the last few years (Luschowet al., 2004).

5. INFECTIOUS BURSAL DISEASE OR GUMBORO DISEASE

Infectious bursal disease virus (IBDV), also known as Gumboro disease, is an acute, highly contagious, and immunosuppressive disease of chickens between 3–6 weeks old, resulting in significant economic losses worldwide (Li et al., 2013, Vukea et al., 2014). IBD is caused by the infectious bursal disease virus (IBDV), an RNA virus that belongs to the genus *Avibirnavirus* of the family *Birnaviridae*. The IBDV has a non-enveloped capsid structure containing a double-stranded RNA genome with A and B segments (Brown and Skinner, 1996, Muller et al., 2003). IBDV termed avian nephrosis or "classic IBDV," was first reported from Gumboro in Delaware, the USA, in the year 1962 (Cosgrove, 1962), and subsequently, the disease has been recorded all over the world (Muller et al., 2003). In India, IBDV was first reported in 1971 (Mohanty et al., 1971), after which a series of outbreaks were reported in different parts of India (Sreedevi and Jackwood, 2007, Juneja et al., 2008, Mittal et al., 2006). Currently, IBDV is endemic and a severe problem for the poultry industry in India. The northeastern part of India shares a porous border with China, Bangladesh, Bhutan, and Myanmar, where new strains of IBDV are regularly reported (Li et al., 2015, Rashid et al., 2013, Islam et al., 2012). 4 different IBDV outbreaks were reported during 2014–15 in the Kamrup, Darrang, and Jorhat districts of Assam (Morla et al., 2016).

Diagnosis can be made based on history (age of affected flock), clinical signs, and a swollen edematous bursa at necropsy is sufficient for diagnosis. Laboratory tests include virus isolation, where the Bursa of Fabricius is the best source of virus isolation during the acute stage of the disease (Anonymous, 2018). The virus can also be isolated in embryonated chicken eggs; Immunofluorescence: Viral antigen can be detected in smears or frozen sections of the bursa using Immunofluorescence; Electron Microscopic examination; Agar Gel Precipitation test: Macerated bursal tissue is suitable for viral detection antigen by AGPT; Virus Neutralization test: Choicest method for measuring IBDV antibodies; ELISA: Macerated bursa tissue is suitable for viral detection antigen by ELISA or by AGPT; Reverse transcriptase PCR can be used targeting VP1 or VP2 genes for characterization of IBDV strains (Anonymous, 2018).

6. MAREK'S DISEASE

Marek's disease (MD) is one of poultry's major economically important viral diseases characterized by rapid-onset lymphoid tumours, ocular conditions (pearl eye), paralysis, and immunosuppression. It is caused by Marek's disease virus (MDV), classified as the gallidalphaherpesvirus 2 (GaHV-2), which belongs to the genus *Mardivirus* of the sub-family *Alphaherpesvirinae*, family *Herpesviridae* (Anonymous, 2017). The MDV genome is a double-stranded DNA (dsDNA) of approximately 180 kilobase pairs, containing two unique regions, the unique long (UL) and the unique short (US) (Bertzbach et al., 2018). MDV has been divided into serotypes 1, 2, and 3 (Bulow & Biggs, 1975). MDV-1 includes all oncogenic strains of these three serotypes, whereas serotypes 2 and 3 include mildly virulent non-oncogenic strains and avirulent strains, respectively (Witter et al., 2005). The Hungarian veterinarian József Marek first identified the disease in 1907 and described it as fowl paralysis, a generalized polyneuritis in chickens. MDV is among the diseases with the highest economic impact in modern poultry production worldwide (Payne and Venugopal, 2000). Overall, the MDV causes economic losses of about $1–2 billion in the poultry industry annually (Morrow and Fehler, 2004). In India, periodical outbreaks of MD were reported in Gujarat (Kalyani et al., 2010), Tamil Nadu, and Karnataka (Raja et al., 2009, Muniyellappa et al., 2013), Uttar Pradesh (Kumar et al., 2018) and, recently, from North-east India (Puro et al., 2018) and Andhra Pradesh (Prathibha et al., 2018). MD outbreaks in India could be associated with the evolution of virulent strains, vaccine breaks, improper vaccine handling, and compromised biosecurity practices.

Diagnosis can be made based on clinical signs such as paralysis of legs and wings, higher mortality levels with lesions of tumours in multiple organs, and enlarged peripheral nerves. Detection of viral- or tumour-specific antigens in tumours by immunohistochemistry is valuable for further confirmation of MD (Gimeno, 2015, Gimeno and Wakenell, 2016). PCR-based molecular diagnostic tests are increasingly being used to detect and quantify viruses in clinical/ farm materials. The availability of nucleotide sequences of several pathogenic and vaccine strains of MDV has enabled the development of sensitive PCR methods for precise detection and quantitation of pathogenic and vaccine strains (Baigent et al., 2016, Kennedy et al., 2017).

7. INFECTIOUS BRONCHITIS

Infectious bronchitis (IB) is a highly contagious disease characterized by neurological and respiratory symptoms,

including dishevelled feathers, depression, and respiratory distress (Wu et al., 2016, Xu et al., 2019). It is caused by the infectious bronchitis virus (IBV), a member of the *Gamma-coronavirus* genus, the *Corona-viridae* family, and the *Nido-virales* order (Walker et al., 2019). IBV is an enveloped, pleomorphic virus with positive polarity (Cavanagh, 2005). Infectious bronchitis (IB) was first described in 1931 in the USA and can be classified into two significant pathotypes based on its tissue tropism, respiratory and nephropathic (Bande et al., 2017). IBV poses a major global economic threat, causing a considerable reduction in the quality and quantity of layer chickens (Cavanagh, 2007, Laconi et al., 2020). In India, IBV isolates have been reported circulating in Maharashtra, Uttar Pradesh, Tamil Nadu, Andhra Pradesh, Orissa, and Assam (Patel et al., 2015, Sumi et al., 2012). More recently, IBV has been reported from broiler chickens in Kashmir, and nephropathic strains were isolated from chickens in Anand, Gujarat (Bayry et al., 2005, Parveen et al., 2017, Patel et al., 2015, Sumi et al., 2012). IBV strains from India mainly belong to genotype-I, lineages 1 and 24, and serotype Massachusetts (Valastro et al., 2016).

Diagnosis can be made based on isolation and identification of the virus from suspected samples by inoculating into 9–10 days old embryonated SPF eggs to produce significant symptoms (Stunting and curling of embryos); Serological tests like Virus Neutralization test; Agar Gel Immunodiffusion and ELISA. Detection and typing of virus isolates can be done using real-time RT-PCR (Anonymous, 2018).

8. AVIAN INFECTIOUS LARYNGOTRACHEITIS

Infectious laryngotracheitis (ILT) is an acute and highly contagious viral disease in chickens, characterized by inflammation and haemorrhage of the larynx and trachea (Craig et al., 2017). Infectious laryngotracheitis virus (ILTV) is a member of the genus *Iltovirus*, within the subfamily *Alphaherpesvirinae*, under the *Herpesviridae* family (Thureen and Keeler, 2006, Davison, 2010). Under electron microscopy, ILTV particles exhibit morphology typical of herpes virions, which consist of a DNA-containing core within an icosahedral capsid closely surrounded by a proteinaceous tegument layer and an outer envelope anchored with viral glycoproteins (Cruickshank et al., 1963, Watrach et al., 1963). This disease causes severe economic losses to the poultry industry worldwide due to increased morbidity, average mortality, decreased weight gain, reduced egg production, and expenses spent on vaccination, biosecurity measures, and therapy to counteract secondary infection by other avian pathogens (Guy and Bagust, 2003, Guy and Garcia, 2008). ILT was described for the first time in 1925 (May and Tittsler, 1925), and since then, the disease has been reported in several countries (Hidalgo, 2003, Chacon et al., 2010). ILT was first reported in India by Singh et al. (1964). Recent reports on ILT outbreaks in India include a field outbreak that occurred in layer farms in Namakkal District, Tamil Nadu, in 2012 (Srinivasan et al., 2012) and a few more reports in the subsequent years (Sivaseelan et al., 2014, Gowthaman et al., 2014, Baksi et al., 2016). Chickens are considered the most susceptible natural host of ILTV in which virulent or reactivated vaccine viruses can cause typical characteristic signs and lesions of the disease.

Diagnosis can be made based on virus isolation and identification of embryonated chicken eggs by the CAM route. Rapid diagnosis includes the demonstration of virus particles in tracheal samples by electron microscopy and the detection of viral antigens in smears or frozen sections by Immunofluorescence; viral antigens can be detected in tracheal samples by ELISA or Agar Gel Immunodiffusion (AGID); PCR is used for differentiation of field strain from vaccinal strains; antibodies to GaHV-1 can be exhibited by virus neutralization test, ELISA (Flockscreen) or AGID (Anonymous, 2014).

9. INCLUSION BODY HEPATITIS

Inclusion body hepatitis disease of young broilers caused by fowl adenovirus created a huge economic loss to farmers in recent years. Clinical signs are non-specific but there is reduced weight gain and a sudden rise in the mortality of birds. The causative organism of IBH is Fowl Adenovirus (FAdV) serotype 4 belonging to group 1 FAdV of the Adenoviridae family (Balamurgan and Kataria, 2004). The fowl adenoviruses (FAdV) group consist of twelve types (formerly serotypes), namely FAdV-1 to 8a, and FAdV-8b to 11 which are classified into 5 different species (A–E) (Zadravec et al., 2013). FAdVs are non-enveloped, 70–80 nm in diameter single linear double-stranded DNA (dsDNA) with icosahedral structure, composed of 252 capsomers. Out of these 252 capsomers, there are 12 vertex capsomers (penton bases) and 240 non-vertex capsomers (hexons). There are seven polypeptides present in the virion capsid. Hexon, as a major protein of the adenovirus capsid, is known to have regions related to virus-neutralizing and serotype specificity (Toogood et al., 1989).

Hydropericardium syndrome (HPS) also known as hydropericardium-hepatitis syndrome/ Angara disease (in Pakistan)/ Litchi heart disease (in India) or inclusion body hepatitis-hydropericardium syndrome (IBH-HPS) caused by aviadenovirus was first described in 1963 in the USA (Helmboldt and Fraizer, 1963). In Asia, the disease was first reported in broiler birds of 3–5 weeks of age from Angara Goth, near Karachi, Pakistan, in 1987 and is therefore commonly known as 'Angara Disease' in Pakistan (Jaffery

et al., 1988, Khawaja et al., 1988, Cheema et al., 1989). In India, HPS was first noticed in the poultry belt of Jammu and Kashmir, Punjab and Delhi during April–July 1994 (Gowda and Satyanarayana,1994). In Assam, the outbreak of the disease has been reported in a few districts since 2017 (Dutta et al., 2021). The disease was mainly characterized by the accumulation of fluid in the pericardial sac and hepatitis, and hence named hydropericardium syndrome. In India, the disease is commonly known as 'leechi disease' due to the characteristic hydropericardium, giving the heart the appearance of the peeled Indian leechi fruit.

Various diagnosis techniques such as gross, serological and molecular techniques came up for IBH detection. Grossly the liver appears pale, friable and enlarged with the presence of focal or diffuse areas of necrosis. The heart shows an accumulation of straw-coloured fluid in the pericardial sac. Commercial ELISA was used for the detection of FAdV antibodies in broiler chicken in 12 districts of Assam where the overall seropositivity recorded was 46.38% (Dutta et al., 2021). Serological tests like agar gel immunodiffusion, counter immune electrophoresis, fluorescent antibody techniques, immunoperoxidase assays and various modifications of ELISA are used for the diagnosis of fowl adenoviral infection in poultry (Khanna et al., 1992, Khehra et al., 1993, Lal et al., 1992). For isolation of the virus in-vivo, in-ovo, and in-vitro in specific pathogen-free (SPF) birds, SPF embryonated eggs, and Chicken embryo liver primary cells respectively (Gulhane et al., 2016). Molecular techniques for the diagnosis of fowl adenovirus such as restriction endonuclease assay (REA), in situ hybridization, using DNA probes, and polymerase chain reaction (PCR) targeting hexon gene can be done (Mittal et al., 2014).

10. CONCLUSION

This review summarizes the evidence for outbreaks of diseases caused by NDV, AI virus, Fowlpox virus, IBDV, MDV, IBV, ILTV and FAdV in poultry in Assam, India. Therefore, it highlights the need to strengthen the levels of poultry disease surveillance and reporting, as well as the need to strengthen the disease diagnostic capacity and preventive measures viz., vaccination and biosecurity. The information related to the presence and circulation of poultry viruses would enable the improvement of the current control and preventive strategies.

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