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A Comprehensive Review on Infectious Bursal Disease Which Has Serious Threat for Ethiopian Poultry Sector

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Abstract
The infectious bursal disease is an acute, highly contagious, and economically important immunosuppressive viral disease of growing chickens which is highly prevalent in most poultry producing regions of the world. The disease is caused by *infectious bursal disease virus*(IBDV*)* which belongs to the genus *Avibirnavirus*, and in the family *Birnaviridae.* The genome of the virus contains two segments of double-stranded RNA which can encode important structural and nonstructural viral proteins. VP2 protein is the major antigen that stimulates host protective immune response and contains independent epitopes accountable for the induction of neutralizing antibodies in chickens. There are two distinct serotypes of the virus which is nominated as serotype 1 and 2. Only serotype 1virus is pathogenic in chickens while serotype 2 virus is nonpathogenic. IBD has become a major constraint in the poultry industry following the reemergence of *IBDV* in the form of antigenic variants that are responsible for a high mortality rate of young chickens. Following the introduction of the disease in Ethiopia, 45-50% mortality rate was recorded in 20-45 days old broilers and layer chickens. Regular outbreaks and the occurrence of novel strains of *IBDV* became a serious threat and challenge to the growing poultry sector in Ethiopia. Therefore, this review article gives updated /current scientific information on IBD virus concerning the pathogenesis, antigenic variants, epidemiology, and different diagnostic techniques to point out the control and prevention direction of the disease. To combile different research outcomes from various sites and the status of the disease in Ethiopia. Finally to highlight the economic significance in the young flourishing poultry industry in the country, and to draw recommendations for effective control and prevention measures.
Keywords: Bursa Fabricius, Infectious bursal disease, Chickens, immunosuppressive, vaccination, Ethiopia
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1. INTRODUCTION

Poultry production is one of the segments of livestock production which is playing significant agricultural activity in almost all developing communities especially in Africa. It is one of the fastest-growing parts of global agricultural demands because it has a peculiar privilege to the sector and improves the living standards of the communities (FAO, 2019). This is mainly due to their quick yield return, which requires low investment, short generation interval, requires small land, and fast reproduction cycle compared to most other livestock (Dessie and Ogle, 2001; Hailemariam *et al.,* 2006). It is one way of getting food and food security to alleviate poverty and malnutrition in developing countries like Ethiopia (FAO, 2019).

Ethiopia has the largest livestock population in Africa. The estimated poultry population of the country is 59.5 million(FAO, 2018). The indigenous chicken population of Ethiopia accounted for about 54,510,523(95.86%). There is 770,052(1.35%) and 1,586,144 (2.79%) exotic and hybrids poultry population respectively (CSA, 2014/15). The contribution of exotic poultry to the Ethiopian economy is significantly lower than in other African countries. The indigenous poultry production supplied 98.5% and 99.2% of the national egg and poultry meat production respectively in Ethiopia (Sebho, 2016). The low economical returns of poultry production are associated with several factors such as disease, poor management, and low genetic potential of the breeds that hinder the productivity of the chickens in most areas of the country. From these problems, outbreaks of infectious bursal diseases and Newcastle diseases are the major reasons for economic loss to the poultry industry(FAO, 2019).

Infectious bursal disease (IBD) or Gumboro disease is an acute and highly contagious immunosuppressive viral disease of growing chickens. The disease is highly prevalent in most poultry-producing regions of the world (Rychlik *et al.*, 2018; Müller *et al*.,2021). The etiological agent of an infectious bursal disease is the infectious bursal disease virus(IBDV). The bursa of Fabricius (BF) of chickens is the primary target organ of the virus (Michel and Jackwood, 2017; Vera *et al*., 2015). The virus primarily infects and destroys actively dividing pre-B lymphoid cells in the bursa of Fabricius. This results in the suppression of birds' immune systems and increases the susceptibility to other avian diseases such as infectious bronchitis, Marek's disease, and Newcastle disease (Apilak, 2006). Only chickens (*Gallus gallus domesticus*) develop infectious bursal disease following infection of the virus. Turkeys, ducks, guinea fowl, and ostriches may also be infected by IBDV but don't show clinical signs (Aliy *et al*., 2020).

IBD virus belongs to the genus *Avibirnavirus*, in the family *Birnaviridae* that has a non-enveloped and icosahedral capsid (Jenberie *et al*., 2014; Aliy *et al*., 2020). The structure of the virus renders the virus to be

resistant to the outside environment (van den Berg *et al*., 2000). The genome of the virus contains two segments of linear and double-stranded ribonucleic acid (A and B segments) with 58-60nm diameter. The viral genome encodes five polypeptides, which are designated as VP1-VP5. The smaller segment B encodes VP1 which has polymerase and capping enzyme activities (von Einem *et al*.,2004; Kumar and Bai, 2018). The larger segment A encodes four viral proteins. These are VP2, VP3, VP4, and non-structural protein (VP5). VP2 and VP3 are the two major structural proteins that form the viral capsid (Jackwood *et al.,* 2018).

There are two distinct serotypes of *IBDV* which are designated as serotypes 1 and 2. The classification of the serotypes is recognized by the virus-neutralizing test. And only serotype one is pathogenic in chickens. Serotype 2 virus is non-pathogenic (van den Berg *et al*., 2000; Vera *et al*., 2015). Serotype 1 has been divided into antigenic subtypes based on antigenic variation and virulence. These are classical virulent strains, very virulent strains, antigenic variants, and attenuated strains (Jenberie *et al*., 2014; Cattoli, 2017). Recently *IBDV* has been also grouped into seven genotypes according to the phylogenic analysis of the hypervariable region of VP2(Cattoli, 2017). The variants were identified first in the United States and later spread to Canada, Newzealand, and Australia(Fernandes *et al*., 2005).

The IBD has been distributed worldwide in all poultry-producing countries (Tesfaheywet and Getnet, 2012). IBD that affects the growing chickens was reported for the first time in and around the area of Gumboro, Delaware, in the United States of America in 1962 (Cosgrove, 1962). Later on, the disease affected most regions of the USA and reached Europe. Currently, the disease is distributed throughout the world and is considered an international problem (van den Berg *et al*., 2000). IBD was reported in Ethiopia for the first time in 2002 involving 20-45 days old broiler and layer chickens from commercial farms *(Jenberie et al*., 2014). Afterward, the disease has become the main concern problem in commercial and backyard poultry production systems in Ethiopia. The natural host of the virus is chickens and turkeys. The virus has been isolated from ducks and other domestic fowls rarely (ICTV, 2012). But, only chickens develop infectious bursal disease following infection by serotype 1viruses. The disease mainly affects chicken at 3-6 weeks of age. The main clinical signs of the disease are enlarged bursa Fabricius, watery diarrhea, and severe depression. The high mortality of young chickens is associated with acute forms of the disease. The specific mortality/death of chickens depends on the virulence of the strain and its dose, age, breed of the animals, and the presence or absence of passive immunity (Qin and Zheng, 2017). Chickens that are less than 3 weeks of age show less acute or subclinical forms of the disease. IBD is transmitted by direct or indirect contact through dropping subjects and contaminated vectors (Flensburg *et al.*, 2010).

IBD has continued as a rigorous problem for the poultry industry following the reemergence of the virus in the form of antigenic variants and hypervirulent strains across the world. Very virulent strain is responsible for high mortality rates of young chickens and severe immunosuppressant that involves both innate and adaptive immune responses. The strains emerged and caused devastating outbreaks that can result in 30% and 60-70% mortality in broiler and layers chickens respectively (Beatriz *et al*., 2004). Then the strains are distributed to the Middle East, Asia, Africa, South America, and Latin America (Mawgod *et al*.,2014). The disease causes either direct or indirect economical loss to the poultry sector. The direct losses are associated with the death of young chickens and the reduction of egg production in pullets (Zachar *et al*., 2016). The indirect economical loss of the disease is resulting from the potential interaction of *IBDV* with other viruses, bacteria, and parasites since it causes virus-induced immunosuppression. These indirect losses are arises due to secondary infections, growth retardation/impairment, condemnation of the carcasses at the slaughterhouse, and failure of vaccine (van den Berg *et al*.,2000; Rauf, 2011; Qin and Zheng, 2017). Following the introduction of the disease in Ethiopia, 45-50% mortality rate was recorded in 20-45 days old broilers and layer chickens (Zeleke *et al*., 2005). The mortality rate of chickens by IBD is 72% in young chickens in the Andassa poultry farm (Jenbreie *et al*., 2013). And 25 to 75% of death linked with *IBDV* outbreak was recorded in exotic and crossbreed chickens (Zeleke*et al.,*2005; Woldemariam and Wossene, 2007). Regular outbreaks and the occurrence of novel strains of *IBDV* became a serious threat and challenge to the growing poultry sector industry in Ethiopia Therefore, the objectives of this review article are:

- to give updated/current scientific information on IBD concerning the pathogenesis, antigenic variants, epidemiology, and diagnostic approach to point out combating direction of the disease
- to compile research outcomes and the status of the disease in Ethiopia
- to highlight the economic significance of the disease in the young flourishing poultry industry in Ethiopia

2. History and distribution of the disease

The infectious bursal disease was discovered as a disease entity in 1957 by Cosgrove in the united states of America (Cosgrove,1962). The first outbreak of infectious bursal disease virus was occurred in and around Gumborro, Delaware, the USA that is why the name Gumboro is given to the disease. It is one of the economically most important diseases affecting the poultry industry worldwide (Touzani *et al*., 2018). Initially,

the disease was described as avian nephrosis since the damage was detected in the kidneys. Later on, the disease was named infectious bursal disease based on morphological and histological changes observed in the bursa of Fabricius (Sali, 2019).

In Ethiopia, IBD was illustrated and detected for the first time in poultry flocks at Debre Zeit in 2002 (Zeleke *et al*., 2002) with high mortality in 20-25 days-old broiler and layer chickens (Zeleke *et al*, 2005). The disease has spread to all commercial farms and multiplication centers. The average occurrence of the outbreak rate ranges from 3-4 farms per year (Touzani *et al.*, 2018). The disease was encountered commonly in both commercial and backyard poultry production systems. Gumboro disease surveillance was conducted by NAHDIC in different regions and the overall prevalence rates were 77.48% (MOA, 2011).

3. Etiology and taxonomic classification

Infectious bursal disease (IBD) is a highly contagious and acute infection of young chickens (*Gallus gallus domesticus*). The virus is very robust and can survive in a wide range of ecological conditions and challenging to commonly used disinfectants (WSU, 2014). *Infectious bursal disease virus (IBDV*) is the etiological agent of Gumboro disease which belongs to the family *Burnaviridae,* the genus *Avibirnavirus (*Rychlik *et al*., 2018). The primary target of the virus is the lymphoid tissue of the bursa of Fabricius. The viral genome contains two-segmented double-stranded RNA that affects chickens, fish, and insects. The family *Birnaviridae* holds three genera that affect chickens, fish, and insects: *Aquabirnavirus*, *Entomobirnavirus*, and *Avibirnavirus*. The infectious bursal disease virus is a type species that infect birds (Robi, 2020).

There are two types of distinct serotypes of the virus that show negligible cross-protection. The two serotypes are illustrated and differentiated by nucleotide sequencing of VP2 gene, virus-neutralization, and cross-protection test assay. Serotype 2 viruses were originally isolated from turkeys but they can also found in other avian species (Daral J Jackwood, 2019; Kegne and Chanie, 2014). Serotype 1 viruses are pathogenic to young chickens whereas serotype 2 viruses are non-pathogenic or immunosuppressive to chickens. Serotype 2 viruses isolated from turkeys and non-pathogenic to both turkeys and chickens. It does not produce immunity against pathogenic strains of serotype 1(Vera *et al*., 2015).

Different strains of serotype 1 viruses have emerged in the United States, Western Europe, and parts of South Asia that were more virulent than older strains in the 1990S (Beatriz *et al*., 2004). Serotype-1 has variations in virulence and pathogenicity that cause disease and immunosuppression in chickens (Kumar and Bai, 2018). Based on antigenic variation and virulence, serotype 1 is further divided into several strains. These are attenuated (vaccine strain), classical strains, variant strains, and very virulent strains of IBDV(Zubair *et al*., 2016). A common characteristic of all IBDV strains is the potential to cause immunosuppression which can lead to devastating losses for the broiler and layer chickens. The amount of damage to the bursa of Fabricius is associated with lymphocyte depletion, inflammation, and reduction of bursa/body weight ratio (Jackwood *et al*., 2008). The classical variants cause bursal damage and lymphoid necrosis which can result in 20-30% mortality (Mawgod *et al*., 2014).

Classical and variant strains are the two major strains within serotype 1. Antigenic drift plays a significant role in the formation of several subtypes within these groups. The hypervariable sequence region of VP2 is a major determinant of antigenic phenotypes of the virus. Single Point mutations in the VP2 hypervariable region contribute to the formation of antigenic drift in *IBDV* which in turn renders currently available IBD vaccine infective (Michel and Jackwood, 2017). The mortality rate of very virulent strains of IBDV is over 50%. VvIBDV strain is capable of infecting chickens in the presence of maternally derived antibodies. The significant economical losses of vvIBDV are associated with high mortality, reduce egg production, increase susceptibility of hosts to other infections (Robi, 2020; Jagoda, 2004). Serotype two antibodies are prevalent in turkeys, ducks, and sometimes found in chickens.

4. Morphology of the virus

The family *Birnaviridae* viruses are non-enveloped, single shelled, and icosahedral symmetry particles with a diameter of 65nm. The capsid of the virus is made up of a single protein called VP2 (ICTV, 2012). The structure of the virus is based on a T=13 lattice and the capsid subunits are predominantly trimer clustered (Dey *et al*., 2019).

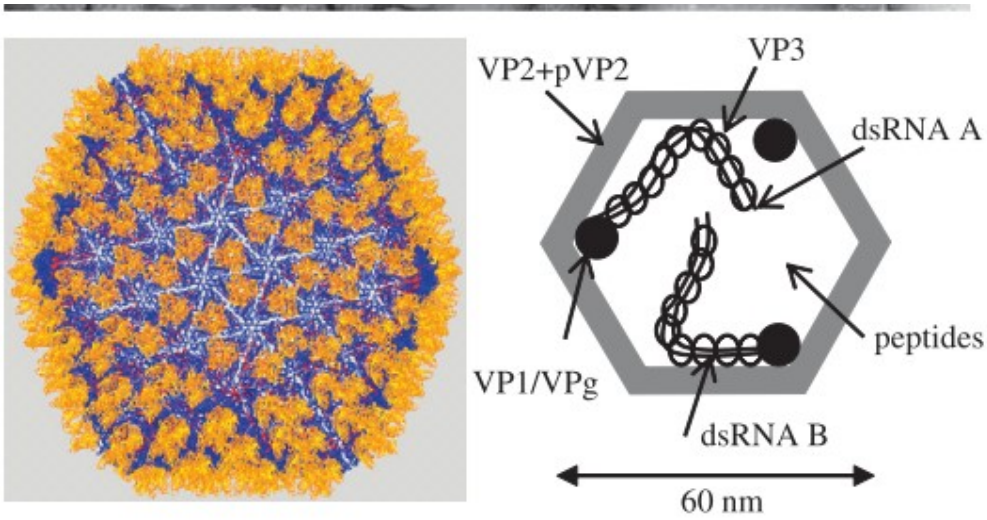

Figure 1. Morphology of IBD virus (ICTV, 2012).

5. Genomic organization

Infectious bursal disease virus is a small, non-enveloped, icosahedral capsid with a diameter of about 60nm virus, which belongs to the family *Birnaviridae* (van Den Berg, 2000). The genome of the virus contains two segments of linear double-stranded ribonucleic acid (A and B segments). The genome encodes five viral polypeptides, which are designated as VP1-VP5. The smaller segment B encodes VP1 which has polymerase and capping enzyme activities (von Einem *et al*.,2004; Kumar and Bai, 2018; Qin *et al*., 2010). The larger segment A encodes two capsid proteins VP2 and VP3, viral protease VP4, and non-structural protein VP5. The non-structural protein (Vp5) cleaved by the viral encoded protease VP4 and yields VP2 and VP3 capsid protein, and VP4 protease (Jackwood *et al.,* 2018). VP2 and VP3 are the two major structural proteins that form the viral capsid.

The structural protein VP2 is the most extensively studied fragment of the viral genome. It is the major antigen that stimulates host protective immune response and contains independent epitopes accountable for the induction of neutralizing antibodies in chickens. The major neutralizing epitope is placed within the hypervariable region (HVR) (Dormitorio, 2007). VP3 has a carboxyl-terminal region accountable for either packaging or stabilizing the RNA genome within the interior of the capsid. It is group-specific antigens and minor neutralizing sites (Nagarajan and Kibenge, 2014).

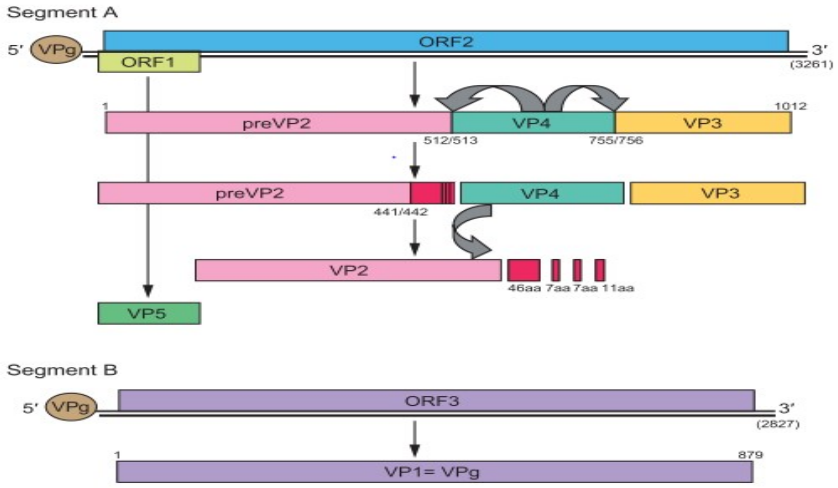

Figure 2. Genomic organization of segments A and B (ICTV, 2012).

VP1 is freely found in the viral particles and covalently associated with the genome as VPg.VP4 is a protease that cleaves its N-and C-termini in the polyprotein and further processes preVP2. A nonstructural, positively charged polypeptide encoded by the small ORF of segment A has been designated VP5 (ICTV, 2012)

6. Epidemiology

Infectious bursal disease has occurred worldwide in poultry-producing countries. The prevalence and spread of the disease is a highly contagious disease of growing chickens that is linked with high mortality and morbidity. 80% of the OIE member countries reported the occurrence of the disease (Teshome *et al*., 2015). Chickens in 3-6 weeks of age are the most vulnerable to clinical disease. The virus is highly prone to mutation, resistant to heat and chemicals. Therefore, the virus can persist in feces, budding, contaminated feed, and water for up to four months in certain conditions. The incubation period is 2-3 days and can be shed the virus within 24hrs following infections (Kegne and Chanie, 2014).

6.1. Host range

The natural host of the virus is chickens and turkeys. The virus has been isolated from ducks and other domestic fowls rarely (ICTV, 2012). Only chickens (*Gallus gallus domesticus*) develop infectious bursal disease following infection by serotype 1viruses. Turkeys, ducks, guinea fowl, and ostriches may also be infected by the virus but don't show clinical signs. Clinical disease is recognized only in young chickens (Teshome *et al*., 2015). Duck can also the asymptomatic carriers of serotype 1viruses. Turkeys are asymptomatic carriers of serotype 2 (Van den Berg *et al*., 2000). This mainly affects chicken at 3-6 weeks of age and has a predilection for the bursa of Fabricius where the virus infects actively dividing and differentiating B-lymphocytes. The high mortality of young chickens is associated with severe acute forms of the disease. Chickens that are less than 3 weeks of age show less acute or subclinical forms of the disease. All breeds are susceptible to serotype 1viruses infections, but the most clinical signs, lesion, and high mortality rate are observed in white leghorns chicken (Mekuriaw, 2015).

6.2. Physico-chemical nature of the infectious bursal disease virus

Infectious bursal disease virus is extremely resistant to adverse environmental conditions and different types of chemicals and disinfectants. It is more resistant to heat, ultraviolet light, ether, chloroform, phenolic derivatives, and quaternary ammonium compounds (Rashid *et al*., 2013). The virus is extremely stable and tends to persist in the environment despite thorough cleaning and disinfection (Sharma *et al*., 2019). The virus is also impervious via exposure for 1hour at 0.5% to 30% phenol and 0.125% trimersal. The infectivity of the virus noticeably decreased when exposed to 0.5% formalin for 6hours. It is also heat stable, and feasible after treatment at 56°C for 5hrs (Teshome *et al.*, 2015).

6.3. Route of excretion and transmission of the virus

Chickens infected with the IBDV shed the virus in their feces that leads to contamination of feed, water, and poultry house. Other chickens found in the house become infected by ingested contaminated feed and water (Kegne and Chanie, 2014). Infectious bursal disease is transmitted by direct or indirect contact through dropping subjects and contaminated vectors. The oral route, upper respiratory tract, and conjunctiva play a significant role in natural infections of the virus (Flensburg *et al*., 2010). The common mode of transmission of the virus is mainly through the oral route and/or horizontal mode of infection is a common route (Orakpoghenor *et al.*, 2019). There is no report on the vertical transmission of the disease. Infected birds excrete the virus in their feces at least for 14 days (Mazengia, 2012; Rashid *et al*., 2013). The resistance of the virus to the external environment increases the indirect transmission potential of the virus.

6.4. Risk factors

The number of chickens in the house, type of breeds, age of chickens, hatchery, and feeding mills are the possible risk factors that enhance the incidence of the disease. The risk of chickens getting clinical disease is strongly associated with the age of parent chickens. Offsprings from older parent chickens are more susceptible to infections compared to offsprings come from younger chickens since antibody level decrease over time as the age of the chickens' increase (Flensburg *et al*., 2010). A higher incidence of the disease is reported in crossbreed chickens while the lowest incidence is recorded in indigenous chickens. There is also a difference in the occurrence of the disease in young and adult age chickens. Production systems, types of strains, environmental and management factors are also associated with the occurrence of the disease (Jenbreie *et al*.,2013).

6.5. Morbidity and mortality

Infectious bursal disease is tremendously communicable among chickens. The morbidity rate of IBD is extremely high and usually about 100% for all strains. Whereas the mortality rate is fluctuating broadly with virulence strains and types of breeds that range from 30% to 60% in chickens infected with classical strains. The fatality rates of vvIBDV strains range from 70% to 100%. The variant strains don't cause mortality in infected chickens, but they severely atrophy bursa(Wu *et al.*, 2007). Only 1-2% specific mortality was recorded by low virulence field strains until 1987. But, the mortality rate of chickens has been increasing in different parts of the

world following 1987(Kebede, 2018). Following the emergence of virulent new strains, a high mortality rate was observed in the USA, Europe, Japan, and other countries. VvIBDV strains emerged and caused devastating outbreaks that can result in 30% and 60-70% mortality in broiler and layers respectively in the mid-1990s. Then the strains are distributed to the Middle East, Asia, Africa, South America, and Latin America(Mawgod *et al*.,2014).

7. ANTIGENIC VARIATION

Antigenic variation is one of the most effective means by which a virus can escape neutralizing antibodies and persist an immune response of the host. Knowing the genetic variation of the virus provides significant insights into the strategies of the virus to use altered structure and facilitate the design of future vaccines (Lana *et al*., 1992). Significant economic losses have been persistent due to the emergence of antigenic variants of *IBDV* (Vakharia *et al*., 1994).

Antigenic drift and genetic recombination contribute to the development of several antigenic subtypes of serotype 1 viruses. The classical strains of viruses are the first *IBDV* strains detected in Gumboro Delaware states. classical and variants indicate antigenic diversity among serotype1 *IBDV*. Partial cross-neutralization and protection are detected between antigenic strains of *IBDV*. However, some strains have significant antigenic drift that results in little or no cross-neutralization or protection between the strains (Jackwood, 2019). Neutralizing monoclonal antibodies bind to variable regions between amino acids 2006 and 350. The variable domain region is highly hydrophobic with a small hydrophilic region present at each terminus. Sequencing of the VP2 gene of several diverse *IBDV* strains and the selection of escape mutants have confirmed that this variable domain represents the molecular basis of antigenic variation (van den Berg, 2000). The high mutational rate of IBD viruses induces high genetic diversity and the frequent appearance of more virulent forms, which can overcome the preceding acquired immunity and persists in the population (Olsen, 2015).

8. PATHOGENESIS

Pathogenesis is the mechanism by which the virus causes injury to the host that results in mortality, disease, or immunosuppression of infected hosts (van den Berg *et al* 2000). The most common mode of infection is via oral route. The IBD affects young chickens at 3-6 weeks of age (Sharma *et al*., 2019). Subclinical infections are demonstrated in older birds. Layer chickens more susceptible to vvIBDV than broiler chickens and higher mortality is recorded in light than heavy breeds. The result of IBDV depends on the type of strain and dose of infection virus, age, breed of chickens, route infection, and presence or absence of neutralizing antibodies (Kebede, 2018). Bursa of Fabricius is the primary organ of the predilection site where most B cells are actively dividing in young chickens. The virus replicates first in the gut-associated macrophages and lymphoid cells ceca and small intestine following the entry of the virus through the fecal-oral route and inhalation. Primary viremia is developed and the virus reaches BF within 11hr post infections. Then it replicates in BF and B cells, the virus enters blood circulation to cause secondary viremia. The virus also spread in other organs like kidney and muscle tissue that directs to pathognomonic clinical signs and death. Degeneration and necrosis of B-cells follicles are observed (Dey *et al*., 2019).

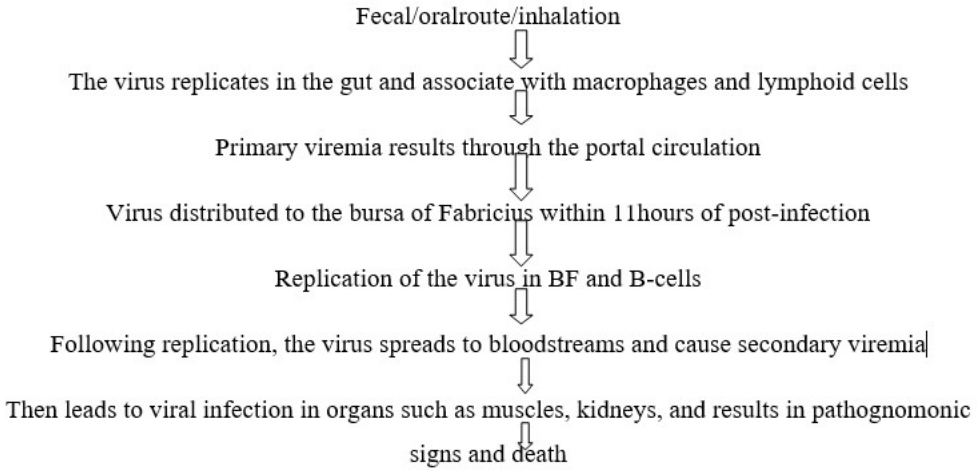

Figure 3. Pathogenesis (Dey *et al*., 2019).

9. DIAGNOSIS TECHNIQUES OF THE VIRUS

The infectious bursal disease can be diagnosed based on clinical signs, the post-mortem examination on BF, serological method, cell culture, and molecular techniques. Chickens less than three weeks of age don't show

clinical signs while chickens greater than three weeks of age present clinical signs (Kegne and Chanie, 2014). Avian coccidiosis, Newcastle disease, and infectious bronchitis are the differential diagnosis of IBD. The occurrence of the bursal lesion allows for the detection of the virus in all acute forms of the disease. Atrophy of bursa in the case of subclinical cases difficult to distinguished from Marek's disease or other infectious anemia. In this case, histopathological observation of the bursa helps to differentiate these diseases (Mekuriaw, 2015; OIE, 2018).

9.1. Clinical signs and post mortem lesions

The infectious bursal disease has clear characteristics of signs and post-mortem lesions. The primary diagnosis of the virus engages flock history, clinical signs, Peak mortality followed by recovery in five to seven days of infections, and post-mortem lesion (necropsy) examination. The pathological lesion that is recognized in the bursa of Fabricius is used as a histopathological diagnosis of the disease (Shegu, 2019; Shafqat *et al*., 2017). The onset of clinical signs of the disease arises after an incubation period of 3-4days in clinical infections. The infections stay for 5-7 days after infection. The main clinical signs of IBD are dullness, depression, ruffled feathers, white watery diarrhea or greenish-yellow diarrhea, and subnormal temperature then the death of chickens recorded (Kumar *et al*., 2018; Zohair et al., 2017). Laboratory diagnosis of the disease depends on the recognition of specific antibodies to the virus or detection of the virus in tissues through immunological or molecular diagnostic techniques. Isolation and identification of the agent give the most definitive diagnosis of IBD (OIE, 2018).

9.2. Histopathology

The bursa specimens for histopathology examination should be dehydrated in alcohol, fix in paraffin, and slice at 4um thickness, then stain by Hematoxylin and Eosin stain for microscopic examination (Mawgod *et al*., 2014; Singh *et a*l., 2015).

Histological diagnosis is based on the demonstration of changes present in the bursa and other organs. The possibility of a diagnosis of both acute and chronic or subclinical forms of the disease is the advantage of histological diagnostic approach. Typical features of *vvIBDV* strains are the ability to cause lesions in the thymus, spleen, or bone marrow (van den Berg *et al*., 2000).

Bursa of Fabricius and other organs demonstrate major diagnostic lesions. Pathological lesion detected in BF includes enlarged and turgid of bursa with yellow discoloration, edematous, swelling, and sporadically hemorrhagic lesions, and finally, atrophy of the bursa of Fabricius examined. Petechial hemorrhages in legs and thigh muscles, splenomegaly, swelling of the liver, and nephrosis are detected in post mortem examination (Mekibib *et al*., 2018; OIE, 2018). The pathological lesion may also be observed in the kidney, intestine, skeletal muscles, and spleen. Congestion and hemorrhage of bursa and skeletal muscles are occasionally observed in vvIBDV. Microscopic lesions that can be observed in bursa are inflammation, lymphocyte necrosis, atrophy of follicles, and follicular depletion of lymphocytes (Olsen, 2015).

9.3. Virus isolation in cell culture

Sample preparation

Bursa of Fabricius should be taken aseptically from five infected chickens in the acute phase of the disease. Slice/cut the bursae using sterile scalpels into smaller pieces. A small amount of peptone broth with penicillin and streptomycin is added to the samples and homogenize in a tissue blender. Then centrifuge thoroughly the mixed specimen at 3000gm for 10minutes. Finally, harvest the supernatant fluid for the detection of the IBDV. The specimen collected from supernatant fluid should be filtered through 0.22µm filter paper to prevent bacterial and fungal contamination (OIE, 2018).

Infectious bursal disease virus may be demonstrated in the bursa of Fabricius samples in the acute stage of infections within the first three days following the manifestation of clinical signs *(Dey et al*.,2019). Isolation of *IBDV* is can be carried out by employing specific antibody-free embryonated chicken eggs, cell culture, and specific pathogen-free embryonated eggs from specific antibody-free sources (Mutinda *et al*., 2015). The replication cycle of *IBDV* takes short time about 4-8hours. Numerous IBDV strains have been adapted to replicate and generate a cytopathic effect in primary cell cultures of the chicken derivation of organs like bursal lymphoid cells, chicken embryo kidney cells(CEK), and chicken embryo fibroblast cells(CEF) (Nagarajan and Kibenge, 1997). The virus does not replicate in kidney cells before it undergoes serial passages in chicken fibroblast cells. Following serial passage on CFC, the virus can produce a CPE in kidney cells in 3-5 days of inoculation (Kebede, 2018).

Inoculate the specimen to recently prepared chicken embryo fibroblast(CEF) cultures that are taken from specific pathogen-free sources in 25cm^2 flasks. Bind at 37ºC for 30-60 minutes and wash it with salt solution two times. The preservation medium is added to each flask. Then observe CPE daily following incubation of the culture at 37°C overnight. If CPE is not detected after 6days of inoculation, the medium should be discarded and

freeze-thaw the cell cultures and inoculate the resulting lysate into new cell cultures. The procedures should be repeated at least three times blindly. If CPE is demonstrated, the virus must be tested against monospecific IBDV antiserum in a tissue culture virus neutralization test. Highly pathogenic strains of the virus should be first pass extensive serial passage in embryos to adapt cell culture and show CPE (OIE, 2018).

9.4. Isolation of virus in chicken embryos

Homogenized specimens of the required amount are inoculated into the yolk sac of 6-8 days old embryonated chickens egg, and on to chorioallantoic membrane of 9-11 days old embryonated eggs originating from chicken free of anti-IBDV antibodies. Five pathogen-free chickens are required to isolate the virus in chicken embryos. Observe inoculated chickens daily and throw away dead embryos 48hours post-inoculation. Embryos that die after 48hrs are examined for lesions. Serotype 1 generates dwarfing of the embryo, subcutaneous edema, congestion, and subcutaneous or intracranial hemorrhage(van den Berg *et al.*, 2000; OIE, 2018). The liver, spleen, and kidneys are swollen and congested. Then the lesions are confirmed by monospecific anti-IBDV serum in embryos. The route of inoculation is CAM, yolk sac, and intra-allantoic. The most sensitive and least sensitive route of inoculation is CAM and an intra-allantoic route respectively (OIE, 2016).

9.5. Serological diagnosis

Blood samples should be collected from the wing vein for serological investigation of the disease. The collected blood samples are allowed to clot via placing in a slant position at room temperature and harvest serum. The serum can also be separated by centrifugation and stored at-20ºC until processed (Sali, 2019). Serological investigation of the virus is carried out using a virus neutralization test, Enzyme-linked immunosorbent assay(ELISA), indirect immunofluorescent test, and agar gel immunodiffusion (AGID). These tests are used for monitoring vaccine responses and diagnosis of infection of unvaccinated flocks (OIE, 2008; OIE,2012). Serological diagnosis has small attention in endemic zones since the tets can't differentiate antibodies provoked by pathogenic IBDV or those elicited by attenuated vaccine viruses. The serology test is also indispensable to verify the disease-free status of flocks. An adequate number of individual serum samples should be taken from the flocks under study (van den Berg *et al*., 2000; Teshager, 2015).

9.5.1. Agar gel immunodiffusion (AGID)

Agar gel immunodiffusion diagnosis method is used to detect the viral antigen in the bursa samples taken from susceptible chickens. A portion of the bursa is taken, bursa specimens cut into pieces, homogenized, and placed in microtiter plates against well-known positive serum (Dey *et a*l., 2019). It is useful in the early stages of infections before the development of antibody response. Freeze-thaw cycles of the minced tissue liberate *IBDV* antigens from the tissue. Agar gel immunodiffusion is the simplest test, but its sensitivity and specificity are low and time-consuming (Kumar *et al*.,2010).

9.5.2.Virus neutralization test

The virus neutralization test is a golden standard diagnostic method and a highly specific and extremely sensitive test for detecting serotype-specific antibodies. VNT test measures the capability of field serum to neutralize the virus and/or prevent CPE of the virus. Virus neutralization test has been done using cell culture. The requirements for undertaken the test are specific pathogen-free CEF cells, appropriate continuous cell lines(Vero or DF1), and adapted variants of IBDV (OIE, 2018).

Principles of VNT: A constant amount of virus is mixed with a different concentration level of serum. Chicken embryo fibroblast cell suspension is added and incubated with serum and virus at 37ºC for 4-5 days. In the end, the plates are fixed and stained with appropriate dye then observe the presence or absence of CPE. The highest dilution of the serum that prevents the killing of CEF cells is taken as the neutralization endpoint of the titer (Wu *et al*., 2007). It is a single serological investigation that differentiates antibodies provoked via the two serotypes and different types of subtypes of serotype 1 strains. It may be also useful for estimating vaccine response. The virus neutralization test is not used as a routine diagnostic since it is labor-intensive, expensive, and gives delayed results (OIE, 2018).

9.5.3. ELISA

ELISA was developed for the detection of IBDV antibodies for the first time. It is the most commonly used diagnostic test for the demonstration and quantification of antibodies to check the efficacy of the vaccination, natural field exposure, and perish of maternal antibody titer (van den Berg *et al*., 2000; Aregitu, 2015). The test is an inexpensive, easy, and rapid test that is carried out a large number of samples at the same time and automated to computer software. There are different types of ELISA such as direct, indirect, competitive, and sandwich ELISA.

Principle of ELISA: The plate is coated with a known antigen of IBDV. Dilution of test serum samples is added and wash to remove unbound antibodies, followed by enzyme-linked secondary antibodies and substrates subsequently. If there is a positive sample, the antibody will bind and react with the substrate. The substrate reaction is stopped by adding an appropriate stop solution. The color reaction is quantified by measuring the

optical density of each well. The ELISA permits the quantification of antibodies to IBDV (OIE, 2008; Sali, 2019).

9.5.4. Immunofluorescent identification

Immunofluorescence tests are used to detect the antigen in bursal tissue using *IBDV* specific chicken antiserum (Kegne and Chanie, 2014). A piece of bursa samples is prepared by microtome cryostat and dehydrated at ambient temperature. It should be fixed with cold acetone. Fluorescent-labeled IBDV-specific antibodies are added to the specimens. Incubate at 37°C for an hour in a moist atmosphere. Wash the mixed specimen and antibody for 30 minutes with phosphate-buffered glycerol saline(PBS) and dipped in distilled water. Finally, examine by UV microscope for IBDV-specific fluorescence (OIE,2008; OIE, 2016). Immune peroxidase staining, direct and indirect immunofluorescent test used to detect viral antigens specific to *IBDV* in the bursal follicles of infected chickens between the 4^{th} and 6^{th} days after inoculation. The use of monoclonal antibodies for the discovery of the virus boosts the specificity of the test (van den Berg *et al*.,2000).

9.6. Molecular identification technique

Classical diagnostic procedures such as serological tests and virus isolation methods are time-consuming, labor-intensive and cannot distinguishes very virulent strains of IBDV from classical strain virus. Molecular diagnostic techniques like RT-PCR, real-time RT-PCR, and nucleic acid hybridization play a significant role in the detection and differentiation of *IBDV* strains viruses than conventional diagnostic tools (Kataria et al., 2001). The most commonly used molecular diagnostic technique in the detection of the viral genome is reverse-transcription polymerase chain reaction (RT-PCR) (OIE, 2018).

9.6.1. RT-PCR

RT-PCR uses RNA as template material for in vitro nucleic acid amplification. The innovation of retroviral reverse transcriptase enzyme in 1970s made the use of RT-PCR possible. Reverse transcriptase is an RNA-dependent DNA polymerase that can catalyze DNA synthesis using RNA as the template. RT-PCR is commonly used in the diagnosis and quantification of RNA virus infections (Wu *et al*., 2007b).

Reverse-transcription polymerase reaction (RT-PCR) is one of the major commonly used molecular diagnostic tests to identify the IBDV genome in the bursa of Fabricius. It helps to detect viral RNA in homogenates of infected organs/embryos and cell culture without taking into account the feasibility of the virus present. It is not required to grow the virus before amplification since RT-PCR can detect the virus genome without replicate in cell culture (Aliy *et al*., 2020). RT-PCR in combination with restriction fragment length polymorphism (RFLP) is a very functional and quick process for characterization and recognition of existing and evolving strains of the virus. RT-PCR products are sequenced for the advanced characterization of IBDV strains. The outer capsid proteins of the VP2 gene contain a hypervariable region that suggests the possible site for the differentiation of IBDV strains (Zierenberg *et al*., 2000).

Three main steps are performed in RT-PCR: extraction of nucleic acid from specimens, transcription of IBDV RNA into complementary DNA (cDNA) using the enzyme reverse transcriptase, and amplification of cDNA by PCR. Transcription and amplification steps require short sequence complementary oligonucleotide primers to target virus-specific nucleotide sequences. The IBDV genome is double-stranded RNA and hence can not be degraded by RNase unlike the ssRNA virus (Sali, 2019). RT-PCR can be carried out in a one-step or a two-step assay. Buffer, reverse transcriptase enzyme, sequence-specific primer, template RNA, and DNA polymerase are put in a single tube. In a single tube, reverse transcription and amplification process is performed. Reverse transcription and amplification processes are carried out in separate tubes in a two-step assay.

Infectious bursal disease virus RNA can be extracted from infected tissues like the bursa of Fabricius using commercially available kits. And /or it can be extracted by adding 1% sodium dodecyl sulfate and 1gm/ml protease K to 70ul of bursal homogenate. Then incubate the mixed material for 60 minutes at 37°C. Finally, nucleic acids are collected by ethanol precipitation and resuspended in RNase-free distilled water or appropriate buffer. The harvested water-diluted RNA must be kept at -20°c until processed (OIE, 2008, Sali, 2019).

9.7. Treatments, prevention and control

9.7.1. Treatments of infectious bursal disease

There are no precise therapeutic agents for infectious bursal disease. Antibiotic is used to treat or prevent secondary infection of the disease. vitamins, electrolytes, and minerals are given as supportive treatments to keep the acid-base balance in equilibrium (Deresse, 2017).

9.7.2. Prevention and control

The high confrontation of the virus to physical and chemical agents is responsible for the persistence of the virus in the exterior environment. Eradication of the disease from affected countries seems impractical. Only vaccines can not resolve the problem unless a combination of strategies is employed (van den Berg *et al*., 2000). Therefore, a successful prevention and control program should involve an effective vaccination program,

hygienic measures, medical prophylaxis, good chick source and effective biosecurity practice. It is virtually difficult to remove all sources of infection since the virus is excreted in large amounts and resistant to external environments and disinfectants. Effective cleaning and disinfection of houses between the flock and all-in all-out management can reduce the challenge of the virus. Formaldehyde and iodophors are the most effective disinfectants of *IBDV* (Teshome *et al*., 2015). The disease is mainly controlled by thorough hygienic measures and immunization through the use of either live or killed vaccines (Aliyu *et al*.,2016). Recombinant and subunit vaccines have been licensed in some countries for the prevention of IBD. The classical live vaccine provides lifelong and broad protection, but it possesses a relative risk of reversion to virulence.

9.7.3. Vaccination of infectious bursal disease

The precise approach for the prevention and control of infectious bursal disease are depending on hygienic management, vaccination schedule, level and variation of maternally derived antibodies, and choice of vaccine strain. And standard vaccination programs and strict biosecurity measures are essential means for the prevention and control of the disease as reported in various studies (Sze *et al*., 2016; Shegu, 2019). The vaccination programs are different depending on several factors. These factors are types of production systems, level of biosecurity, level of maternally derived antibodies, availability of vaccine, costs and potential losses due to IBD.

Infectious bursal disease vaccine has been prepared only for serotype 1 viruses. Hosts infected with serotype 2 viruses can stimulate the production of antibodies even if it does not cause clinical disease like serotype 1 viruses. Antibodies produced by Serotype 2 viruses don't give protection against serotype 1 infection. Serotype 1 viruses have different descriptions of antigenic variants. There are four major types of vaccines existing for the prevention and control of infectious bursal disease. These are live attenuated vaccine, immune complex vaccine, live recombinant vectored vaccine, and inactivated oil-emulsion adjuvanted vaccines (OIE, 2018; Mahgoub, 2012).

Live attenuated vaccine

The vaccine can be produced from completely or partially attenuated classical and variant strains of *IBDV* through passaging the viruses in tissue culture or embryonated chicken eggs (Daral J. Jackwood and Sommer-Wagner, 2011). The classical live attenuated vaccines are appropriate for mass vaccination, and that can provoke strong humoral and cellular immunity in 3 to 6 weeks of age of chickens (Negash and Rautenschlein, 2016). It can be mild, intermediate, or intermediate plus vaccine based on the degree of attenuation of vaccine strain and ability to cause varying degrees of histological lesions. Serotype 1 vaccine strains do not cause mortality, but they can cause various levels of bursal lesions range from mild to moderate or severe (Sze *et al*., 2016).

Mild or intermediate vaccines are used in parent chickens to produce a primary reaction before immunization. They should be administered after all MDA has been diminished since the vaccines are susceptible to MDA. MDA can protect the chickens against early immunosuppressive infections for 1 to 3 weeks of age. The protection of MDA may also be extended to four to five weeks by enhancing the immunity in breeder groups with oil-adjuvanted vaccines (Eterradossi and Saif, 2008). The route of administration of the vaccines are intramuscular injection, spray or using drinking water at eight weeks of age (OIE, 2018). Mild vaccines are sound for specific-pathogen-free chickens for vaccination of breeder flocks. It is not extremely effective in the existence of a high level of maternal antibodies or against vvIBDV strains (Camilotti *et al*., 2016). Therefore, a mild vaccine should be administered after maternally derived antibodies have been disappeared, that is between the fourth and eighth weeks of age (van den Berg *et al*., 2000).

Broilers and pullets chickens can be vaccinated using intermediate or intermediate plus vaccine strains. If young chickens are at high risk of natural infection with virulent IBD, intermediate vaccines are used to protect against infection. The vaccine may be administered to day-old chickens by nebulisation to protect chicks from infection even if the vaccine is susceptible to MDA. The route of administration of the vaccine is by drinking water or spray, intramuscular and eye drop is used. Live infectious bursal disease vaccine causes bursal damage and interferes with the response of other vaccines. Therefore, only healthy chickens ought to be vaccinated (OIE, 2008; OIE,2018).

Immune complex vaccine

The immune complex vaccine can be produced from a combination of live *IBDV* vaccine and IBDV- specific antibodies. The vaccine is given in the hatchery by in–ovo-injection at 18days of incubation. It can be also given to day-old chicks in the hatchery. The challenge of MDA has been overcome and chicks are effectively immunized(OIE, 2018).

Live rccombinant vaccine

Live recombinant vaccines use a viral vector to express the VP2 antigen of *IBDV* in chickens. The vaccine is presently licensed in many countries globally. It is produced for in-ovo-or day-old use(OIE,2018). The problem of MDA and compatibility with other diseases have been overcome by live recombinant vaccines (Le Gros *et al.*, 2009, Lemiere *et al.*, 2011). The antibody response stimulated by live recombinant *IBDV* vaccines expressing the VP2 protein may contain antibodies directed against VP2 only. Virus neutralization test can be detected

neutralizing antibodies against VP2 protein (Müller *et al.*, 2012).

Inactivated IBD vaccine

The produced vaccines have high, lifelong, and consistent levels of antibodies in breeding chickens that have formerly been vaccinated with live vaccine or exposed by natural infection to field virus during rearing. The live vaccine should be administered first at about 8 weeks of age which is followed by inactivated vaccine at 16-20 weeks of age. Sometimes, inactivated and live vaccines may be given mutually for young valuable chickens with high MDA levels raised in areas with a high risk of exposure to virulent IBDV to increase the effectiveness of the vaccine. The ideal route of administration of the vaccine is intramuscular at leg muscle or subcutaneous route (OIE, 2012; OIE, 2018).

10. Status of infectious bursal disease(IBD) in Ethiopia

Infectious bursal disease is an acute, highly contagious, and economically important immunosuppressive viral disease of growing chickens. It is one of the well prevalent viral diseases of young chickens in different parts of Ethiopia (Rychlik *et al.*, 2018; Müller *et al.*,2012; Zeleke *et al.*, 2005). The disease is a recently emerging disease of chickens in Ethiopia as reported by Zeleke *et al*., (2005). The disease is considered to be introduced simultaneously with the increased number of commercial, state, and private poultry farms in the country (Zeleke *et al*, 2005). It is causing a threat to the young flourishing poultry industry in the country since different researches and case reports are coming from different regions of the country (Mekuriaw *et al*., 2017).

Infectious bursal disease outbreaks affecting 20-45 days-old broiler and layer chickens were explored in Ethiopia for the first time in 2002. The fatality rate of IBD in different poultry farms ranges 45 to 50% with a mean fatality of 49.89%. The mortality rate of the disease in broiler and layer chickens is 58.09% and 25.08% respectively as studies showed (Zeleke *et al*, 2005). It also results in a reduction of both the figure and productivity of the sector. There is a difference in disease incidence among age, production systems, and breeds of the chickens as different studies indicated. The seroprevalence of IBD is significantly higher in the cross and exotic breed compared to local chickens (Tadesse and Jenbere, 2014). The highest incidence of the disease is found in intensive production systems(85.9 %) than extensive production systems(81.6 %). The mortality rate of young chickens in Andassa poultry farm due to IBD is 72% (Jenbreie *et al*., 2013). And 25 to 75% of death recorded in exotic and crossbreed chickens over the past few years linked with *IBDV* (Zeleke *et al.,*2005; Woldemariam and Wossene, 2007).

Infectious bursal disease is extensively distributed all over the country and constraints of all types of production systems and different breeds of chickens as studies reported. The cause of disseminating the disease to indigenous chickens is associated with the distribution of improved breeds of chickens from infected breeding and multiplication centers. The over all seroprevalence of IBD in the country is 83.1% (Jenbreie *et al*., 2013).

Table 1. Seroprevalence IBD in Ethiopia

Study area	Seroprevalence of IBD	RT-PCR confirmation	Source
West Gojam	75	Not confirmed	Kassa and Mollab, 2012
North Gondar	72	Not confirmed	Kassa and Mollab, 2012
North Shewa of Amhara	96.2	Not confirmed	Girma *et al*., 2017
Debre Zeit	93.3%	Confirmed	Zeleke *et al.,*2005
	82.2	Not	Tesfaheywet and Getnet, 2012
North Shewa Oromia	73.8	Not	Girma *et al*., 2017
Mekele	45.05	Not	Sinidu et al.,2015
Jjiga and Harar	51.7%	Not	Girma et al., 2017
Eastern shewa	83	Not	Tadesse and Jenbere, 2014
Andassa poultry farm	100%	Not	Woldemariam and Wossene, 2007
Wanji	92.1	Not	Tadesse and Jenbere, 2014
Selected site of Ethiopia	83.1	Not	Jenbreie *et al*., 2013
Bishoftu, AA, Kombolcha, Assela and sululta	NA	confirmed	(Shegu *et al*., 2020)
In and around Bahir dar (Bahir Dar city, Andassa, Meshenty, Wonjeta and Gombat)	51.6	Not	Teshager, 2015

11. Conclusion and recommendation

The infectious bursal disease is one of the major viral diseases of growing chickens across the world, including in Ethiopia that causes significant economic losses in poultry sectors. The disease is an acute, highly contagious,

and economically important immunosuppressive viral disease of growing chickens which is highly prevalent in most poultry producing regions of the world. The disease is caused by *IBDV* which belongs to the genus *Avibirnavirus*, and in the family *Birnaviridae.* The genome of the virus contains two segments of double-stranded RNA which can encode important structural and nonstructural viral proteins. The disease has continued as a rigorous problem for the poultry industry following the reemergence of the virus in the form of antigenic variants and hypervirulent strains across the world. The disease is introduced simultaneously with the increased number of commercial state and private poultry farms in Ethiopia. It is a highly prevalent viral disease of young chickens in different parts of Ethiopia that prone to a threat to the young flourishing poultry industry in the country. The disease causes either direct or indirect economic losses in both commercial and village chicken production systems. The characteristics of the virus are extremely resistant to adverse environmental conditions and different types of chemicals and disinfectants. The high mutational rate of IBD viruses induces high genetic diversity and the frequent appearance of more virulent forms, which can overcome the preceding acquired immunity and persists in the population. Therefore; the following recommendations are forwarded: Regular studies should be undertaken for the identification and characterization of currently circulating viral strains to design cost-effective vaccines. The antigenic and genetic diversity of the virus variants circulating in the country within regular periods should be done. Epidemiological investigation of the disease and regular vaccination and biosecurity measures are appropriate for the control and prevention of the disease in the country.

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