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REVIEW ON DIAGNOSTIC AND VACCINATION APPROACHES OF INFECTIOUS BURSAL DISEASE OF POULTRY.

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A PAPER SUBMITTED TO DEPARTMENT OF VETERINARY LABORATORY TECHNOLOGY IN PARTIAL FULFILLMENT OF THE COURSE CURRENT TOPICS IN VETERINARY MICROBIOLOGY

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LIST OF ABBREVATIONS

Ab	Antibody
AGID	Agar Gel Immunodiffusion Test
BF	Bursa Of Fabricious
CPE	Cytopathic Effect
ELISA	Enzyme Linked Immunosorbent Assay
IBD	Infectious Bursal Disease

IBDV	Infectious Bursal Disease Virus
Ig	Immunoglobulin
MDA	Maternally Derived Antibody
OD	Optical Density
OIE	International Animal Health Organization
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
RFLP	Restriction Fragment Length Polymorphism.
RT PCR	Reverse Transcription Polymerase Chain Reaction
SPF	Specific Pathogens Free Chickens
VIBD	Virulent IBDV
VNT	Virus Neutralization Test
VP	Virus Protein

SUMMARY

Infectious bursal disease, also known as the Gumboro disease is a highly contagious and acute viral disease of poultry characterized by destruction of lymphoid cells.Diagnosis of Infectious bursal disease involves consideration of the flocks' history, clinical signs and lesions. Objectives of this paper are to highlight various commonly used diagnostic method of Infectious bursal disease and to review advances made in diagnostic method and Vaccination strategies for Infectious bursal disease, with special emphasis on the strengths and weaknesses of each of those techniques. Isolation of Infectious bursal disease virus followed by its serological assay and histopathological examination of bursa is regarded as the gold standard method of Infectious bursal disease diagnosis. Serological tests such as Agar gel immune diffusion, Enzyme linked immuno sorbent assay and Viral neutralization test are commonly used laboratory assay in diagnosing Infectious bursal disease virus.

Recently most accurate and relatively fast diagnostic method, Molecular technique are widely used. From the reviewed diagnostic technique Molecular diagnostic technique is relatively the easiest and sensitive one. The virus causes immunosuppression where if the infected chicken recovered from the acute disease, they become more susceptible to infections of other pathogens. Therefore, prevention is important and vaccination has become the principal control measure of Infectious bursal disease Virus infection in chickens. Conventional attenuated live and killed vaccines are the most commonly used vaccines. With the advancement of knowledge and technology, new generation or genetically-engineered vaccines like Deoxyribonucleic acid and sub unit vaccines have been used. Different vaccination strategies like in ovo, at hatch and post hatch vaccination are implemented. Hatchery vaccination is becoming a common practise. Based on this review paper more safe and effective Infectious bursal disease vaccines that are affordable and readily available must be identified with further cost benefit analysis.

Keywords: *Infectious bursal disease, infectious bursal disease virus, diagnosis, vaccine, vaccination.*

INTRODUCTION

Infectious bursal disease (IBD), also known as Gumboro disease, has been a great concern for poultry industry worldwide. It was first reported from broiler flocks in the area of Gumboro, Delaware in 1957 (Liew *et al.*, 2016).

Infectious bursal disease is caused by Infectious Bursal Disease Virus (IBDV) which is acute and very contagious virus, affects growing chickens between the ages of 3 to 6 weeks (Saidi *et al.,* 2020). It is caused by a virus that is a member of the genus *Avibirnavirus* of the family *Birnaviridae (*OIE, 2008), which is characterized by destruction of lymphocytes in the bursa of Fabricius (Mutinda, 2016). It is non-enveloped, double-stranded RNA and bi–segmented virus, that is, segment A and B (Eterradossi and Saif, 2008). Two serotypes of IBDV are identified namely the serotypes 1 and 2. The serotype 1 is pathogenic in chickens and consists of three viral strains namely the classical (ca), very virulent (vv) and variant (va) IBDV. The serotype 2 is non-pathogenic in chickens (Liew *et al*., 2016).

Infectious bursal disease (IBD) is commonly encountered lymphocytolytic disease that adversely affects the defense mechanism of birds and results in immunosuppression and failure to develop satisfactory immunity (Getachew and Fesseha,2020).

Infectious bursal disease virus infections clinical signs, organ lesions and immuno-suppression correlate with the status of immunity, age and genetic background of affected chickens and with the virulence of the infecting virus strain After an incubation period of 2-3days, young chickens show symptoms of ruffled feathers, watery diarrhea,trembling, severe prostration, severe depression, vent picking, presence of urate stains on the vent, dehydration, loss of appetite and elevated water consumption, and also death may follow 1-3 days later. Mortality will peak and recede usually in a period of 5-7 days (Zahid *et al.*, 2016).

Generally a preliminary diagnosis can usually be made based on flock history, clinical signs and post- mortem (necropsy) examinations. Necropsy examination will usually

show changes in the bursa of Fabricius such as swelling, oedema, haemorrhage, the presence of a jelly serosa transudate and eventually bursal atrophy (Aregitu, 2018).

Various diagnostic methods like virus neutralization test (VNT), enzyme linked immunosorbent assay (ELISA) and agar gel immunodiffusion test (AGIDT) are used limitedly to detect IBDV and molecular techniques like reverse transcriptase polymerase chain reaction (RT-PCR) have frequently used to detect viruses from the field samples (Mathivanan *et al.*, 2004). Laboratory confirmation was achieved by virus isolation followed by its serological assay and histopathological examination of affected bursa (Yousif, 2005). The virus isolation is laborious, nonspecific and time consuming. The more frequently used molecular method is the reverse-transcription polymerase chain reaction (RT-PCR) (Wu *et al.*, 1992).

The main effective way to control IBD is vaccination and different vaccination programmes are regularly implemented globally including Africa (Vandenberg, 2000). Vaccination has become the principal control measure of IBDV infection in chickens since the virus is resistant to different physical and chemical method of decontamination (Liew *et al*., 2015). Vaccines and vaccination programs vary widely depending on several local factors (e.g. type of production, level of biosecurity, the local pattern of disease, the status of maternally derived antibodies, vaccines available, costs and potential losses(Getachew and Fesseha,2020).

The objectives of this paper are:

- To highlight various commonly used diagnostic method of Infectious bursal disease.
- To review advances made in diagnostic method and Vaccination strategies for IBDV, with special emphasis on the strengths and weaknesses of each of those techniques

1 INFECTIOUS BURSAL DISEASE

Infectious bursal disease is an acute and highly contagious viral infection of immature chickens. IBD is characterized by destruction of lymphocytes in the bursa of Fabricius and to a lesser extent in other lymphoid organs. Infectious bursal disease virus is an etiology of infectious bursal disease "Gumborodisease" (Mahgoub, 2012; Muller *et al.*, 2012).

1.1 Etiology

Infectious bursal disease virus is a double strand RNA virus (dsRNA) and a nonenveloped, icosahedral capsid with bi-segmented genome (Wu *et al.*, 2007).The larger segment, A, is 3261nucleotides long and contains two open reading frames (ORF) and encodes four viral proteins designated as VP2, VP3, VP4 and VP5 and also the smaller segment B encodes only VP1 which has polymerase activity. The two viral proteins, VP2 and VP3 are structural proteins which form the viral capsid. The epitopes responsible for the induction of neutralizing and protective antibodies are located on the VP2 protein (Wagari, 2021).

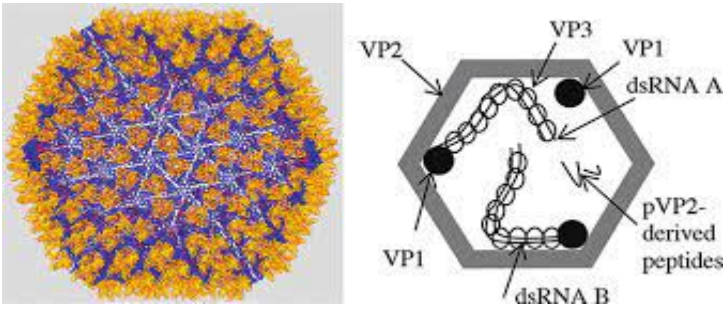

Figure 1 : Structure of infectious bursal disease virus particles

Mutations in the IBDV genome have impacted antibody recognition and led to variations in antigenicity, immunogenicity, virulence, and tropism of circulating infectious bursal disease virus strains (Zierenberg *et al.*, 2004).

1.2 General characteristics of the Infectious Bursal Disease Virus

Two serotypes of Infectious bursal disease, serotype one and two have been recognized as having considerable antigenic variation within each serotype (Jackwood *et al.*, 2018). It is a naked virus, devoid of envelope, known by its resistance to physical and chemical agents and resistant to pH conditions of 2–11 but it is inactivated at pH 12. Due to this ability of stability and hardiness, it persists in poultry premises even after thorough cleaning and disinfection for up to 4 weeks in the bone marrow of infected chickens. The virus has been shown to remain infectious for 122 days in a chicken house, and for 52 days in feed, water and faeces (Lukert and Saif, 2003).

1.3 Pathogenesis of Infectious Bursal Disease

Following host entry via oral ingestion or inhalation, IBDV may bind to host cell proteins such as N-glycosylated polypeptide(s) expressed on the cell membrane of immature IgM+ B-cells during viral entry process. It is transported by infected macrophages to the bursa of fabriciaus where the virus undergoes intra cytoplasmic replication in IgM+ B lymphocytes (Orakpoghenor *et al.*, 2020). Due to its short incubation periods which range from 2 to 3 days a pore forming peptide of the virus (pep46), which is associated with the outer capsid of the IBDV particle, may facilitate viral entry into the cytoplasm of infected cells (Yip *et al.*, 2012).

The mature and competent lymphocytes will expand as a result of stimulation by the virus, whereas the immature lymphocytes will be destroyed. The bursa is infiltrated by heterophils and undergoes hyperplasia of the reticulo endothelial cells and of the inter-follicular tissue (OIE, 2004).

1.4 Diagnosis

Diagnosis of Infectious bursal disease involves consideration of the flocks' history, and of the clinical signs and lesions.Clinical manifestations and post mortem findings of affected birds may aid to diagnose IBD disease but laboratory diagnosis is necessary for

its confirmation (Banda, 2002). Chickens less than 3 weeks of age present no clinical signs of disease but chickens greater than 3 weeks of age present clinical signs (Kegne and Chanie, 2014).

Gross and histopathological examinations of the bursa are used to diagnose IBD in young chickens or in those having maternal antibodies (Lukert and Saif, 2003). However, other methods used in diagnosis include isolation and detection of IBDV using embryonated chicken eggs, cell culture, reverse transcription polymerase chain reaction (RT-PCR) and serology, such as virus neutralisation, indirect ELISA and agar gel immune diffusion test (Brandt *et al*, 2001).

1.4.1 Virus Isolation

IBDV can be isolated (grown) on chicken embryo and primary cell culture especially chicken fibroblast cell. Isolation and identification of the agent provide the most certain diagnosis of IBD, but are not usually attempted for routine diagnostic purposes as the virus may prove difficult to isolate (Yousif, 2005).

I. Isolation of virus in embryos

The inoculation of bursal homogenates from IBDV infected chickens per the chorioallantoic membrane of 9-10 days old embryonated SPF (Specific-pathogen-free) chicken eggs is the most sensitive diagnostic method for virus isolation. The most sensitive route of inoculation is the Chorio allantoic membrane; the yolk sac route is also practicable(OIE, 2012). It is important especially for Wild-type IBDV, usually not replicating in conventional cell culture, can also be regenerated by the reverse genetics approach, but can grow in embryonated chicken eggs (Brandt *et al.*, 2001). Some strains grow well in embryos but are not readily adapted to grow in Chicken embryo fibroblasts or Chicken embryo kidney. Variant viruses however, do not kill the embryos but cause embryo stunting, discoloration, splenomegaly and hepatic necrosis (Lukert and Saif, 2003).

II. Isolation of virus in cell culture

IBDV grows in chicken embryo fibroblast and produces CPE characterized with an appearance of round retractile cells in about 3-5days (Orakpoghenor *et al.,* 2020).IBDV isolation in cell culture is not a routine use as a diagnostic test because the virus is difficult to culture. Some field strains failed to grow on cell cultures (Bumstead *et al.,* 1993). Wild–type Infectious bursal disease virus strains particularly very virulent strain do not grow in tissue culture. Comparison of genome sequence of wild-type and tissue culture adapted IBDV strains pointed to several mutations that might be responsible for invitro growth of IBDV in tissue culture (Islam, 2002).

1.4.2 *Serological identification*

For serological investigations, usually blood can be collected from the wing vein, allowed to clot and serum separated by centrifugation and stored at -20 °C until tested. Serological tests generally used for the detection of IBDV are Agar Gel Immuno diffusion test, ELISA and VN (Viral neutralization test) (Shaima *et al*., 2014).

A. Agar gel immunodiffusion test(AGID)

The AGID is the most useful of serological tests for detection of specific antibodies in serum or for detecting viral antigen in bursal tissue (OIE, 2004). The test is specific because it cannot give false positive results, but it can give a false negative result. The presence of IBDV antigen can be detected in the bursal tissue by AGID for 5- 6 days post infection (Murphy *et al*., 1999).

Blood samples should be taken early in the course of the disease, and repeat samples should be taken 3 weeks later. Because the virus spreads rapidly, only a small proportion of the flock needs to be sampled. Usually 20 blood samples are enough. For detection of antigen in the bursa of Fabricius, the bursae should be removed aseptically from about

ten chickens at the acute stage of infection. The bursae are minced using two scalpels in a scissor movement, then small pieces are placed in the wells of the AGID plate against known positive serum. Freeze– thaw cycles of the minced tissue may improve the release of IBDV antigens from the infected bursal tissue(OIE,2008).

B. Virus neutralization tests

VN tests are carried out in cell culture. The test is more laborious and expensive than the AGID test, but is more sensitive for detecting antibody (OIE,2004). This sensitivity is not required for routine diagnostic purposes, but may be useful for evaluating vaccine responses or for differentiating between IBDV 1 and 2 serotypes. To reduce test-to-test and operator-to-operator variation, a standard reference antiserum may be included with each batch of tests and the titer of the virus suspension must be reassessed in each new experiment using a sufficient number of repeats (wells) per virus dilution (OIE,2008).

C. Enzyme-linked immunosorbent assay

The enzyme linked immune sorbent assay (ELISA) is the most commonly used test for the detection and quantification of IBDV antibodies to check response to vaccination, natural field exposure and decay of maternal antibody titer. It is economical, simple, and quick tests a large number of samples at the same time and is adaptive to automation to computer software (Lukert and Saif, 2003).

The test sera are diluted according to the established protocol or kit instructions and each is dispensed into the requisite number of wells. After incubation under the appropriate conditions, the sera are discarded from the plates, and the wells are washed thoroughly. Anti-chicken immunoglobulins conjugated to an enzyme are dispensed into the wells, and the plates are again incubated as appropriate. The plates are emptied and rewashed before substrate containing a chromogen that gives a color change in the presence of the enzyme used is added to the plate. After a final incubation step, the substrate/chromogen reaction is stopped by addition of a suitable stopping solution and the colour reactions are

quantified by measuring the optical density of each well. The Sample to Positive (S/P) ratio for each test sample is calculated (OIE,2008)

1.4.3 Identification by molecular method

Molecular detection and characterisation, involving sequencing, and phenotypic and genotypic analyses have been utilised in the diagnosis of IBD. This method can detect the genome of IBDV, which is unable to grow in cell culture or embryonated eggs because it is unnecessary to grow the virus before amplification even when the virus is present in a very minute quantity and has lost its infectivity (Mittal *et al.,* 2005).The classical methods for molecular characterization and differentiation of IBDV field isolates include reverse transcriptase polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP), nucleotide sequence analysis, and quantitative real time RT-PCR (qRT-PCR) (OIE,2008).

Reverse Transcriptase polymerase chain reaction offers a rapid, highly sensitive and specific test for the confirmative diagnosis of the disease which would help in controlling the disease, thereby reducing the economic losses significantly. RT-PCR in combination with restriction enzyme analysis allows the rapid identification of vvIBDV. Nucleotide sequencing of RT-PCR products is widely used for further characterization of IBDV strains (Zierenberg *et al.,* 2004).

The VP2 gene of IBDV contains variable region which suggests the potential of this region for differentiation of IBDV strains. RT-PCR followed by digestion with multiple restriction enzymes or RFLP and nucleotide sequencing of VP2 gene have been used for differentiation of IBDV strains. The molecular differentiation of IBDV strains using VP2 has been improved by use of labeled probes in real-time RT-PCR (Jackwood, 2005).

1.4.4 Post mortem findings

Pathological change observed at the bursa of fabricius is characteristic and histopathological investigations combined with the demonstration of viral antigen by

immunohistochemistry confirm an IBDV infection (Zeleke *et al*, 2005).Diagnostic lesion includes muscle hemorrhages and bursal enlargement . Pathognomonic gross lesions observed in the bursa of fabricius which show doubling in size with a yellowish gelatinous film that may surround it and sometimes hemorrhages may seen on the surface of it (Liew *et al*.,2016).

Figure 2: gross lesions observed in the IBDv affected bursa of broiler chicken.

1.4.5 Histopathology examination

The lymphoid structures primarily affected by IBDV are BF, spleen, thymus, Harderian glands, caecal tonsils, gut-associated lymphoid tissue (GALT) and head-associated lymphoid tissues (HALT). Lymphocytic degeneration and necrosis in the medullary region of the BF at 1 day post infection are the first signs (Getachew and Fesseha, 2020).

Microscopic examination of tissues shows moderate hemorrhages in the muscles and kidneys and the spleen shows moderate lymphoid depletion in the lymphoid nodules. There is marked interfollicular oedema and depletion of 13 lymphocytes from the lymphoid nodules in the BFs. Other lymphoid nodules of the BF show degeneration and necrosis of lymphocytes and cystic cavitations with heterophil infiltrates.(Eterradossi and Saif, 2008).

1.5 Vaccine and Vaccination against IBD

Vaccination of chickens with high quality vaccines is the primary method of control of many poultry infectious diseases including IBD (Gumboro) disease (Dacic, 2008).

It becomes possible to safeguard chicken with a proper vaccination schedule. Rational vaccination schedules and strict biosecurity measures were indicated in many reports as essential tools for the control of IBD (Farooq, 2003). Even though there are different types of IBD vaccine being developed two of them are commonly used for the control of IBD. These are live attenuated vaccines, or inactivated oil-emulsion adjuvanted vaccines (Aregitu, 2018). Currently plant based and a live recombinant vaccine expressing IBDV antigens has also been licensed (Wagari, 2021)

1.5.1 Live-attenuated vaccines

Live viral vaccines can trigger immune system in the target host. They can replicate and induce both cellular and humoral immunity. They do not require an adjuvant to be effective and are suitable for mass administration to the chicken, but they may also have undesirable side effects. These include horizontal and vertical transmission (although the latter not in the case of IBD vaccines), reversion to virulence and vaccine reactions that may result in disease or production loss. In general, the live IBDV vaccines in use by the poultry industry have been attenuated by serial passage in tissue culture, eggs or embryo-derived tissues, with the aim of maintaining the immune response induced by the parent virus whilst attenuating the ability of the vaccine virus to cause clinical disease or significant immunosuppression (Schijns *et al.,* 2008).

1.5.2 Inactivated vaccines

Inactivated IBD vaccines are mostly formulated as water-in-oil emulsions, usually combining several antigens and have to be injected into each bird. It has been observed that inactivated IBD vaccine were able to induce IBDV-specific T-cell and inflammatory

responses in chickens (Rautenschlein *et al.*, 2005). It has been reported that inactivated IBD vaccines must have either a high or an optimized antigenic content in order to induce in breeders an immunity that helps protect the progeny from infection by variant IBDV strains (Muller *et al*., 2012).

Killed-virus vaccines in an oil adjuvant are often used to boost levels of maternal antibodies and Confer longer lasting immunity in breeder hens. The duration and uniformity of this immunity may be influenced by the concentration and antigenic specificity of the vaccine strain. These vaccines are not ideal for stimulating a primary antibody response; therefore, they tend to be most effective in chicks that have been "primed" with a live virus vaccine or naturally infected through field exposure to IBDV (Eterradossi and Saif, 2008). Currently, many oiladjuvant vaccines contain both classic and variant IBDV strains. Killed-virus vaccines are administered by subcutaneous or intramuscular injection at sixteen to twenty weeks of age (Van den Berg , 2000).

1.5.3 New generation or Genetically-Engineered IBD vaccines.

Genetically engineered IBD vaccines have also been developed as a result of improved understanding on the molecular structure and immunology of IBDV. The viral capsid protein VP2, encoded by genomic segment A and derived from a large precursor protein VP0 by a series of proteolytic processes, carries immune determinants that control antibody-dependent neutralization and protection. Generally, these could be divided into two main categories, reflective of their replicative nature upon delivery into the chicken (Delmas, 2008).

1. Non-replicative IBD vaccines

Immunisation by DNA or subunit vaccines involves the use of non-replicating IBDV for induction of immune response in birds. DNA vaccination is based on direct inoculation of plasmid DNA encoding a target immunogen gene into subjects of study (Oshop, Elankumaran and Heckert, 2002). Under the influence of a mammalian promoter, the

target genes were expressed to produce proteins in vivo that are able to induce immune responses in the injected host. Repeated injections of DNA vaccines carrying the IBDV genes, either the polyprotein genes or gene of VP2 alone were shown to protect the chickens from challenge virus (Chen *et al*., 2011).

However, the presence of MDA could affect the efficacy of DNA vaccines and a high dose of DNA vaccines was required to overcome the interference of MDA and induce immune response in chickens. It was shown that a booster vaccination with inactivated IBD vaccine after priming with DNA vaccine provided better and higher protection to the chickens compared to injection with DNA vaccines alone (Hsieh *et al.,* 2010).

2. Replication-competent IBD vaccines

Replication-competent viral vectors have been utilized to express and deliver immunogens of interest to chickens. In contrast to DNA and subunit vaccines, vaccination by live recombinant virus vectors employed the use of live and replicating virus to produce IBDV antigen upon in vivo infection. They have been shown to elicit both humoral and cell-mediated immune response in the chickens. As they could persistently infect the chickens, the potential of having a long-term protective immunity is high (Tsukamoto *et al.,* 2002).

Besides, the recombinant viral vectors are less sensitive to MDA and could therefore evade the neutralisation by the maternal anti-IBDV antibody (van den Berg, 2000). Several viruses have been experimented to express the VP2 protein of IBDV. This includes fowlpox virus, fowl adenovirus, Marek's disease virus, Newcastle disease virus, and avian adeno-associated virus among others (Tsukamoto *et al.,* 2002). The VP2 protein expressed in vivo from these various studies have been shown to confer from partial to full protection to vaccinated chickens from mortality, although they do not prevent the damage to the bursa (Zhou *et al.,* 2010).

1.5.4 Plant-Produced IBD vaccines

The plant-based expression system is a growing alternative platform for production and development of animal vaccines (Liew and Hair-Bejo, 2015). Being one of the pathogenic agents of importance in poultry, plant-based expression system using the stable (Wu *et al.*, 2004), transient (Gómez *et al.*, 2013), or chimeric viral particles (Chen *et al.*, 2012) approach has been used to produce IBD vaccine containing VP2 capsid protein. Transgenic rice expressing the VP2 protein was shown to protect the chickens from challenge following oral immunisation (Wu *et al.*, 2007).

Recently, the VP2 protein of IBDV has been transiently expressed in *Nicotiana benthamiana* leaves and extracted for subunit vaccination in chicken (Gómez *et al.*, 2013). The recombinant VP2 protein emulsified in oil adjuvant, injected intramuscularly to chicks at 18 days of age and followed by booster doses after 22 and 35 days, were shown to induce the production of anti-IBDV antibody with neutralising ability (Chen *et al.*, 2012).

1.5.5 In ovo Vaccination and post hatch vaccination

Recently, technology has been developed to deliver live vaccine into eggs during the incubation period. Live vaccine virus is blended with IBD antibody and the complex is injected in ovo at 18 days of incubation. The eggs go on to hatch and the vaccine virus is released when the chicks are about 7 days of age. In this way, the problem of maternally derived IBD antibody is overcome and the chicks are effectively immunized (OIE, 2012).

Compared to Post-hatch vaccination, In ovo injection of a live intermediate vaccine allowed faster recovery from bursa lesions although both methods exhibited similar protection against challenge (Rautenschlein and Haase, 2005). Although in ovo delivery of vaccines is an attractive alternative to post-hatch vaccination, various factors including the dosage, virulence, and efficacy, among others must be properly optimized before chasing large scale vaccinations (Corley and Giambrone, 2002).

2 CONCLUSIONS AND RECOMMENDATIONS

Infectious bursal disease is caused by IBD Virus that affects immune cells of chickens. It is mainly disease of young chickens between 3-6 weeks old and cause secondary problems due to the effect of the virus on the bursa of Fabricius. Diagnosis of IBD is depending on clinical signs, differential diagnosis, gross lesions, histopathological lesions, virus isolation, serological and molecular diagnosis. Isolation and identification of the agent can deliver the most confident diagnosis of Infectious bursal disease.

From the recommended serological tests for IBD virus, AGID is the simplest but least sensitive whereas ELISA is a rapid and sensitive method but cannot differentiate serotypes. Virus neutralization test is the golden standard and the only serologic test that differentiates antibodies of two serotypes and sensitive but it is more laborious and expensive than AGID. Molecular Identification Reverse Transcription-Polymerase Chain Reaction is used to detect IBDV without considering the viability of the virus by working on VP2 found on segment A of the viral Capsid.

Vaccination is the principal control measure of IBDV infection in chickens. From the available vaccine, live vaccine is more protective and most widely used IBD vaccine. Vaccination strategies in ovo, at-hatch or on-farm vaccinations, determines the choice of vaccines used in the farm. Therefore based on the above conclusion the following recommendations are forwarded:

- Virus neutralization test is the most sensitive but laborious and time consuming
- Molecular diagnostic technique reverse transcriptase polymerase chain reaction is is relatively the easiest and sensitive one. .
- More safe and effective IBD vaccines that are affordable and readily available must be identified with further cost benefit analysis.
- In Ivo vaccination will be the promising best vaccination strategy against Infectious bursal disease.

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