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REVIEW OF INFECTIOUS BURSAL DISEASE (GUMBORO)

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Fulfillments for the Requirement of the Bachelor Degree in Veterinary
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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ

وَرَبُّكَ الْأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

سورة العلق

آية (1-5)

الإهداء

إلى صاحب الخلق العظيم (محمد صلى الله عليه وسلم)

إلى الذي يسعدني في اهلامي ويرافق روحي

في يقظتي (والذي)

إلى التي يشرق وجهها كالقدر الساطع في ليلة صافية

من ليالي الصيف (والذي)

إلى العيون البريئة التي تنظر الي

بجهد (أخوتي)

إلى الشموع التي اضاءت طريق

العلم (أساتذتي)

إلى الذين واكبوا معي طريق سنين العمر

بإخلاص (أصدقائي)

شكر وتقدير

يسرني ويشرفني أن أقدم بخالص شكري وتقديري إلى عمادة
كلية الطب البيطري- جامعة ديالى للجهود التي بذلتها للطلبة.
وأشكر صاحب الفضل استاذي الدكتور (عامر خزعل العزاوي)
لإشرافه على هذا البحث ، فقد أعطاني من وقته وجهوده العلمية
الشيء الكثير في إعداده فجزاه الله خير الجزاء.

ولا يفوتني ان أشكر أصدقائي الذين كان لرفقتهم الطيبة دعما
معنويا لأكمال دراستي وفقهم الله ومن الله التوفيق.

REVIEW OF INFECTIOUS BURSAL DISEASE (GUMBORO)

**By
Rabee Jalil**

Abstract

Gumboro Disease or Infectious Bursal Disease (IBD) is an acute, highly contagious viral infection of young chickens in the world. In the clinical acute form (vvIBDV), the disease causes significant immunosuppression lead to economic losses due to mortality, reduced performance and increased susceptibility to other diseases. The IBD virus is extremely resistant to environmental conditions and most of chemicals. Therefore the control of the disease must take into program of effective vaccination with consideration strict biosecurity. The following review knowledge about the disease, the causative agent, the clinical signs and the role of different elements of the immune system. Infectious bursal disease (IBD) is caused by a virus that belonged to the family birnaviridae. Although turkeys, ducks, guinea fowl and ostriches may be infected, clinical disease occurs solely in chickens. Only young birds are clinically affected. Severe acute disease of 3–6-week-old birds is associated with high mortality, but a less acute or subclinical disease is common in 0–3-week-old birds. This can cause secondary problems due to the effect of the virus on the bursa of Fabricius.

This study describes the IBD which included the virus shape, virus biology, epidemiology, pathogenesis, clinical sings of the disease, gross lesion, histopathology, prevention and control of the IBDV. This study was designed to be a review for researchers in the field of the study of IBDV.

دراسة تفصيلية عن مرض الكمبورو في الدواجن

اعداد

ربيع جليل

مرض الكمبورو أو مرض التهاب غدة فابريشيا المعدي في الدواجن، عبارة عن مرض فايروسي حاد شديد الامراضية في اغلب الحالات ويحدث خسائر اقتصادية في قطاع تربية الدواجن وخاصة في المناطق الكثيفة التربية. يسبب المرض خسائر ونسبة هلاكات مرتفعة وخاصة في القطعان غير المحصنة . يعتبر مرض الكمبورو من اهم امراض الدواجن واكثرها انتشارا ويؤدي الاصابة بالمرض وخاصة في الاعمار الصغيرة الى تثبيط مناعي شديد مؤديا الى جعل الطيور ضعيفة المناعة والمقاومة للعوامل الممرضة الاخرى ويرفع من قابليتها للاصابة بالامراض الفايروسية الاخرى وكذلك يجعل الطيور المصابة قليلة الاوزان قياسا بمثيلاتها من الطيور غير المصابة. تهدف الدراسة الى استعراض واسع لاهمية المرض وكذلك للاعراض السريرية والتغيرات المرضية والنسجية الخاصة بالمرض وطرق الوقاية من المرض وبرامج التحصينات المتبعة وكذلك تهدف الدراسة ان تكون كمرجع بحثي نظرا للمعلومات الواسعة التي تم التطرق اليها لتكون كمرجع لبقية الباحثين فيما يتعلق بمرض الكمبورو

Introduction

The production and consumption of eggs and poultry meat has been increasing worldwide over the last three decades as the consumption of eggs has doubled and that of chicken meat has tripled (Jordan and Pattison, 2001). With increasing industrialization and intensification of rearing systems, the disease pattern in fowl is changing. In developing countries, the infectious diseases still play a predominant role in deciding the economic returns from the poultry industry(1).

Poultry industry has expanded rapidly over the last fourth decades and is playing a vital role in the economy of the country. However the industry is challenge with a variety of problem, particularly the disease of viral origin as most important diseases that causes Varsity losses.

Infectious bursal disease (IBD) also known as Gumboro disease is an acute highly contagious viral infection of immature chickens. Chickens of age 3 to 6 weeks are most susceptible to clinical infection (2). The causative agent is Infectious bursal disease virus (IBDV) with primary affinity for actively mitotic B-lymphocytes within the bursa of Fabricius where it multiplies. In the process of infection other lymphoid organs such as cecal tonsils and spleen are also affected but to a lesser degree (2). Viral activity leads to immune-suppression thereby making chickens susceptible to other diseases and subsequently drops in

egg production and quality and impaired growth of young chickens which results significant economic losses in the poultry industry (3).

The disease was first reported in USA in 1957 and become relatively under control due to proper vaccination programmed both in the hens and chicks. However, in late 1980's outbreak of the disease with high mortality due to very virulent IBD virus (VVIBDV) was reported in Europe (4) and the disease spread worldwide too many countries including Malaysia in 1991(5). In contrast, the disease with high mortality (50%) was happened due to very virulent IBD virus (vvIBDV) in Iraq in 1977 on different region (6).

The disease in a fully susceptible chicken flock, occur at 3 to 6 weeks of age and economic impacts of the disease are manifold including losses due to morbidity and mortality (7).

The causal agent of IBD is infectious bursal disease virus (IBDV), a non-enveloped double stranded RNA (dsRNA) virus. IBDV belongs to the genus Avibirnavirus and family Birnaviridae divided into two serotypes (1 and 2). Serotype 1 is further sub- divided into six (6, 7) subtypes that ranges from a pathogenic to very virulent strains for chickens while serotype 2 have been reported to be non-pathogenic (8).

The disease is manifested by debilitate, dehydration and the development of depression with watery diarrhea, swollen and blood

stained vent (9). Severity of the signs depends on the virus strain and the age and breed of the chickens (10). Infection with less virulent strains may not show obvious clinical signs but the birds may have fibrotic or cystic bursa of Fabricius that become atrophied prematurely (before six months of age) and may die of infections by agents that would not usually cause disease in immunocompetent birds (10, 11). The postmortem findings were hemorrhages in the thigh/pectoral muscles, enlarged, edematous and hyperemic bursa or atrophic in chronic cases and hemorrhage in the junction between gizzard and proventriculus (12, 13).

The disease still remains a problem in the Middle East and Southeast Asia and many regions in the world, in contrast, the disease with high mortality (50%) due to very virulent IBD virus(vvIBDV) was reported in different regions in Iraq(14, 15).

The objective of this study was to describe the incidence, epidemiology, pathogenesis, clinical signs, gross lesion, immunity and different methods that used for vaccination of chicks against IBDL.

Literature review

Definition of the disease

Gumboro Disease or Infectious Bursal Disease (IBD) is one of the most common diseases of commercial poultry in Asia. In the clinical acute form (vvIBDV), the disease causes significant economic losses due to mortality, reduced performance and immunosuppression that lead to increased susceptibility to other diseases. The IBD virus is extremely resistant to environmental conditions and chemicals. Therefore the control of the disease must take into consideration strict biosecurity combined with an effective vaccination program (16).

Clinically the disease is seen only in chickens older than 3 weeks but can happen in chickens less than 3 weeks. The feathers around the vent are usually stained with feces containing plenty of urates. The period of most apparent clinical symptoms and high death rate is at the age of 3 – 6 weeks. IBD could however be observed as long as chickens have a functioning bursa (up to the age of 16 weeks). In chickens younger than 3 weeks, IBD could be subclinical, but injured bursa leads to immunosuppression (17).

History

Early studies to identify the etiological agent of IBD were clouded by the presence of Infectious bronchitis virus (IBV) in the kidneys of field cases.

IBD was first described as a specific new disease by Cosgrove in 1962 and was referred to as “avian nephrosis” because of extreme kidney damage found in birds that succumbed to infection with synonym “Gumboro disease” because of its geographical location. Identification and distinctions between Infectious bronchitis virus and the viral agent responsible for causing Gumboro disease down by (18) and later (19) reported that IBDV infections at an early age were immunosuppressive. In 1970 (20) later proposed the term “Infectious bursal disease” as the name of the disease causing specific pathognomic lesions in BF. Since then, IBDV has been found to be widely distributed in poultry populations worldwide.

Incidences of clinical IBD has been found increasing in the past decade, generally occurring in birds between 21 and 35 days of age with high mortality. However, in earlier outbreaks, low mortality rates were reported. This capability of IBDV infection greatly demanded the attention of research community to work out control strategies for this infection. The signs of IBD as well as pathological changes occurring in lymphoid organs lead to peak mortality occurred at four to six days after onset of the disease (21). Whereas (22) reported that IBD in subclinical form was associated with a variant IBDV which varied in ability to cause mortality but invariably caused immunosuppression. The existence of a second serotype of IBDV was reported in 1980 (22).

IBD has been prevalent as an acute disease of poultry in China since the

first isolation of IBDV in Beijing in 1979, and the outbreaks of IBD had been reported in almost all the poultry rearing parts of the world(23).

In 1986, a very virulent (vv) strain of IBDV (vvIBDV) was isolated in Netherlands. Since then, very severe clinical outbreaks with high mortality rates (90%-100%) caused by vvIBDV have been reported in many countries.

Chickens vaccinated with classical IBDV vaccines were not protected against these new “variant” strains, and succumbed to immunosuppressive disease. At the end of 1980s and during nineties, very virulent strains of IBDV (vvIBDV) were reported in Europe and Asia. Unlike the variant strains, the vvIBDV were antigenically very similar to the classical strains but had a marked increase in virulence causing high mortality in infected flocks (24).

The author had suspected that the outbreak was either due to heavy challenge or increased virulence of the field virus. Antigenic variation among IBDV caused vaccination failures when these variants no longer coincided with the antigenic structure of the vaccine strains commonly used in USA (25).

In 1987/88, vvIBDV strains capable of causing 30 to 70 per cent mortalities in broilers and layers were isolated in Holland, Belgium and UK. Since then, outbreaks of vvIBDV have occurred in most European countries as well as Africa, Japan, China and South East Asia. VvIBDV were able to break through the maternal as well as active immunity induced mainly by classical or mild IBDV vaccines (24).

The Etiology of Disease

When Infectious bursal disease (IBD) appeared in chickens in 1962, the disease was designated as “Gumboro disease” after the geographic location of the first recorded outbreak. Infections caused by IBD virus may exacerbate infections with other etiological agents that lead to reduce chicken’s ability to respond the vaccination program (26).

Infectious bursal disease virus (IBDV) is a non-enveloped virus, with a single capsid shell of icosahedral symmetry composed of 32 capsomers and a diameter of 55 to 60 nm. The virus, IBDV, is a member of the *Birnaviridae* family, whose genome is made of two segments of doublestranded RNA. The virus has five proteins recognized as VP1 to 5. The virus genome consists of two segments, A and B. Segment A (3.2 kbp) codes for two structural proteins, VP2 and VP3, and for two non-structural proteins, VP4 and VP5. Segment B codes for nonstructural protein VP1, the viral transcriptase (27).

Two serotypes, 1 and 2, of IBDV have been reported, of which the strains belonging to serotype 1 are pathogenic to chickens, while those of serotype 2 are nonpathogenic. VP2 of IBDV is considered the major host-protective virus antigen containing at least three neutralizing epitopes and the sites that determine the virus virulence (28, 29).

The variable region of VP2 gene comprises a tight cluster vulnerable amino acid variation, which may be responsible for generation of antigenic variants

among the strains This region, referred to as hyper variable (HVR), is therefore an ideal site for determining the antigenic variation by sequence analysis of different IBDV strains. Molecular characterization of differences among the isolates from different geographical regions may help in developing a correct and effective vaccine and in understanding the evolution of viruses. The target organ of IBDV is the bursa of Fabricius (BF), which is a specific reservoir for B-lymphocytes in avian species at its maximum development. Recovery from disease or sub-clinical infection leads to immunosuppression with undesirable consequences if infection occurs early in life (30).

Studies in our laboratory using the VN test have indicated that there are currently two major antigenic types of IBDV circulating in the US. These are Serotype 1 classic and variants, and a variety of subtypes of both the antigenic types are commonly encountered. These two antigenic types have also been shown by cross-protection studies to be the major immunogenic types of the virus. Infections by viruses of Serotype 2 do not provide any protection against challenge by viruses of Serotype 1. Classic Serotype 1 viruses will provide partial protection against variant Serotype 1 viruses. The magnitude of that partial protection is dependent on the strain and titer of the challenge and vaccine viruses. Variant viruses provide complete protection against variants and classic viruses (31).

Distribution

Infectious bursal disease emerged in 1957 as a clinical entity responsible for acute morbidity and mortality in broilers chickens.

The first report of a specific disease affecting the bursa of Fabricius in chickens was made by Cosgrove in 1962. The first outbreak due to the classical IBDV (calIBDV) were observed in the area of Gumboro and was initially described as avian nephrosis, in Delaware (United States of America) which is the origin of the name, although the terms 'IBD' or 'infectious bursitis' are more accurate descriptions. It was characterized by flock morbidity of 10-25% and mortality averaging 5% (20). Between 1960 and 1964, the disease affected most regions of the USA, and reached Europe in the years 1962 to 1971(16). The condition spread rapidly and was recognized throughout the U.S broiler and commercial egg production areas by 1965. From 1966 to 1974, the disease was identified in the Middle East, southern and western Africa, India, the Far East and Australia. Infectious bursal disease is currently an international problem: 95% of the 65 countries that responded to a survey conducted by the Office International des Epizooties (OIE) in 1995 declared cases of infection, including New Zealand which had been free of disease until 1993. These findings led to the adoption of a specific resolution of the International Committee of the OIE during the 63rd General Session in May 1995 (2).

The first experiment to isolation the etiologic agent was impeded by a lack of specific-pathogen-free (SPF) eggs and by deficiencies in viral and serologic

techniques. By 1967, the highly infectious nature of the agent was recognized. Dependable methods were developed to isolate the virus in embryonated eggs and to adapt it to tissue culture. The agent was characterized as a virus belonging to a new taxonomic group in 1976(16).

The immunosuppressive property of IBD virus was first recognized in 1970 and was confirmed in structured trials in 1976. An early method of control involved planned infection of chickens (16).

Transmission

IBD has been an economically significant, widely distributed condition affecting flocks of chickens. The causal virus is transmitted laterally by direct and indirect contact between infected and susceptible flocks (32), but not transmitted vertically by transovarian rout (16). Indirect transmission of virus most probably occurs on fomites (feed, clothing and litter) or through air, whereas no evidence of egg transmission of thr virus and no carrier state has been detected in chickens (33).

Infected chickens shed IBDV at one day after infection and can transmit the virus for at least 14 days but not exceeding 16 days (32). The virus can remain viable for up to 60 days in poultry house litter , in addition, rodent, wild birds and insects including mites may be playing an important role in transmission of IBDV. Beside, the lesser meal worm was recognized as a carrier and the virus

has been isolated from mosquitoes and evidence of infection in rats has been reported but there is no indication that either species is reservoir for virus (33). In contrast many studies showed that the dog could eventually carried the IBDV after eating infected chickens either by voluntarily or accidentally.

Clinical Signs and Gross Lesions

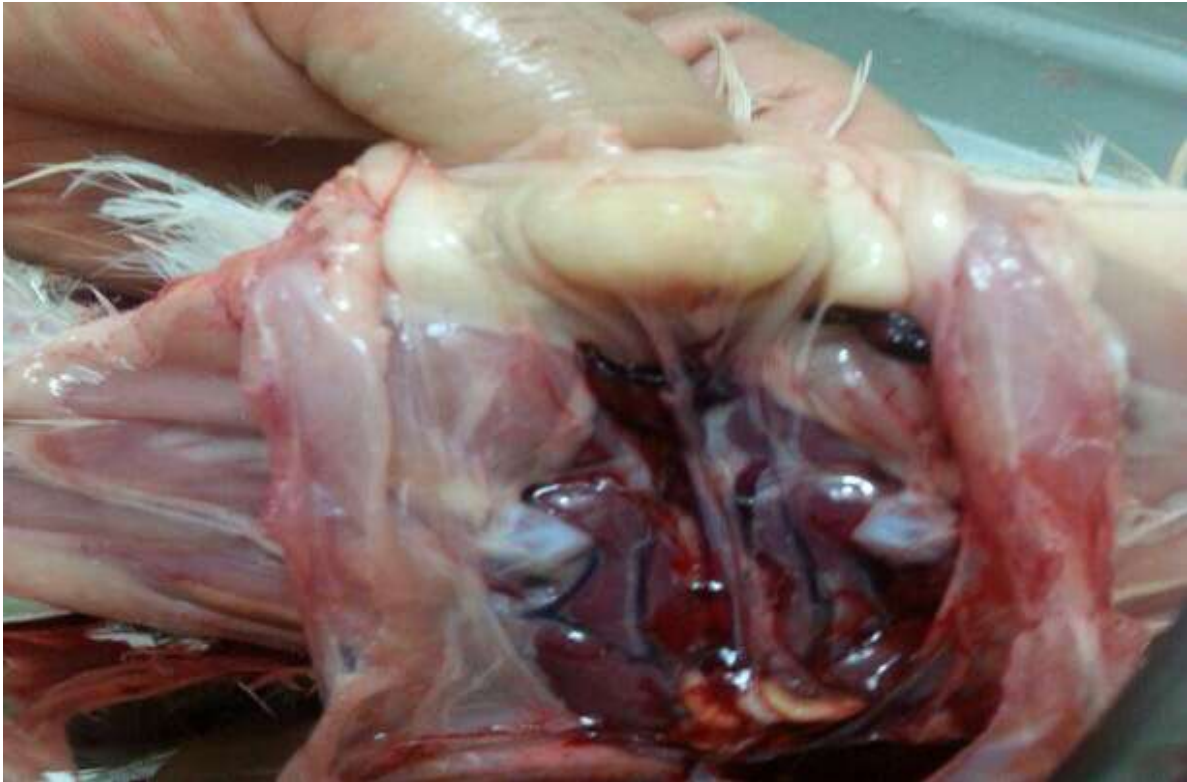
The incubation period of IBD range 2-4 days. The infection of susceptible broiler or layer pullet flocks is characterized by acute onset of depression. Chickens are averse to move and peck at their vents (20) (Pic, 1). In acute outbreaks, the chick appear sleepy and have a reduce food intake. Terminally, birds may show sterna recumbence with coarse tremor and most of the birds have ruffled feathers, droopy appearance and may be seen pecking at the vent. Morbidity and mortality begins 3 days pi, peaks and recedes in a period of 5-7 days. White or watery diarrhea, sold vent feathers and vent pecking are seen. The feathers are ruffled, the birds have an unsteady gait and may become prostrate and trembling prior to death (16).



Picture 1: Infected birds are depressed, have ruffled feathers, droopy appearance and may be seen pecking at the vent.

The short duration of clinical signs and mortality pattern are considered to be of diagnostic significance in IBD (32).

Affected flocks showed depression for 5-7 days during which mortality rise rapidly for the first two days then declines sharply as clinical normality returns. There is usually 100% morbidity, but the mortality varies depending on the virus strains. Clinical signs alone are not sufficient to make a diagnostic, but when combined with gross lesion; it is possible to arrive at preliminary diagnosis (33). Changes in lymphoid organs are typical of the disease. The bursa of Fabricius which is the main target of the virus undergoes major changes beginning at 3days post infection (pi, 2). It increases in size reaching twice the normal size by 4 days pi followed by atrophy, and reaching one third of its original weight by 8 days pi (33) as show in picture 1.



Picture 2a: Showed enlargement of Bursa Fabricius after 3 days pi.

This picture belongs to the broiler chick from case bring to the lab of department of avian pathology, college of veterinary medicine, University of Diyala.

By day 2 or 3 post-infection, the bursa usually has a gelatinous yellowish transudate covering the serosal surface, as shows in (Picture 2a,b). Also longitudinal striations became prominent and the color changed from white to



Picture 2b: Infected birds with Gumboro virus lead to enlargement of bursa Fabricius

creamy. The transudate disappeared as the bursa returned to its normal size and the organs turned gray during the period of atrophy (16). Infected birds are dehydrated; Petechial hemorrhages with darkened discoloration are present in the thigh and pectoral muscles and this hemorrhages (Picture, 3) also reported from the mucosa at the juncture of the proventriculus-gizzard and on the serosal surface and bursa.



Picture 3: Showed Petechial haemorrhages with darkened discoloration are present in the thigh and pectoral muscles of infected bird. This picture belongs to the broiler chick from case bring to the lab of department of avian pathology, college of veterinary medicine, University of Diyala.

The tissue distribution and severity of the lesions is dependent on the subtype and pathogenicity of the virus.

Pathogenic changes in the spleen and thymus were less prominent than those of the bursa (20). The spleen might be slightly enlarged and usually had small gray uniformly dispersed on the surface (Picture, 4)



Picture 4: Enlargement of the spleen of infected bird with Gumboro

Lesions in these organs are noticed at the same time as the changes occurred in the bursa. These lesions resolved within 1 or 2 days of appearance.

The vvIBD infections are characterized by severe clinical signs, high mortality and a sharp death curve followed by rapid recovery. The vvIBD strains have the same clinical signs and incubation period of 4 days as classical viruses but the acute phase is exacerbated (34). The vvIBD strains cause more severe lesions in the cecal tonsil, thymus, spleen and bone marrow. It has been shown that the pathogenicity of the field strains of IBD correlate with lesion production in non-bursal lymphoid organs. The result also suggests the pathogenicity of IBDV may be associated with virus antigen distribution in non-bursal lymphoid organs. Chickens affected by the variant IBDV are characterized by severe bursal atrophy and immunosuppression (35) without showing inflammation induced symptoms associated with the infection of IBDV. Attenuated strains have been adapted to chick embryo fibroblast cells or other cell line. These strains do not cause disease in chickens, and therefore some of them are being used as live vaccines.

Pathogenesis

Pathogenesis is the process through which the virus causing injury to the host leading to mortality, disease or immunosuppression. The different pathogenesis of IBDV has different degree of pathogenicity. The natural infection is usually via the oral route accompanied by the gut associated lymphoid cells(36).

Following oral inoculation of IBDV in susceptible birds, the virus replicate primarily in the macrophage and lymphoid cells of the gut-associated lymphoid tissue during 4-6 hours post inoculation and lead to primary viremia. Then the virus travel to the liver via portal vein and localized in the bursa of Fabricius as the target organ via blood stream where IBDV replication will occur at 13 hours post inoculation (37). After massive replication in the follicle of the bursa of Fabricius, the virus will be released into the blood as secondary viremia. This will be followed by virus replication and destruction to another organ such as cecal tonsil, spleen, bone marrow, gut associated lymphoid tissue and also replication in bursa of Fabricius (36, 37). Consequently, clinical sign and mortality occur within 48 to 72 hours. The cause of death in clinical IBDV is mainly due to circulatory failure as a result of severe hemorrhages. Severe dehydration owing to diarrhea and reduce water intake could also lead to circulatory failure and death.

Haemorrhage in IBDV infected chicken can be due to impairment of the clotting mechanism due to destruction of thrombocyte (38) and depletion of

haemolytic component. In addition haemorrhages can also be the result of formation of immune complexes culminating to an arthus reaction.

Microscopic lesion particularly in the bursa of Fabricius is similar to an Arthus reaction, which is caused by deposition of antigen antibody complement complexes which in turn induces production of chemotactic factors, haemorrhage and leukocytes infiltration(38). Two week old chicks showd less circulating complement than 8 weeks old chicks and did not show the Arthus reaction . In addition, IBDV infected chickens showed prolonged clotting time, which has consequently induced hemorrhagic lesions in the birds(38).

The target organ of IBDV is the bursa of Fabricius at its maximum development, where B lymphocytes mature in avian species. Bursectomy can prevent illness in chicks infected with virulent virus. Actively dividing, surface-immunoglobulin-M-bearing B cells are lysed by infection but cells of the macrophage lineage can be infected in a persistent and productive manner and play a crucial role in the dissemination of the virus. The severity of the disease is directly related to the number of susceptible cells present in the bursa of Fabricius; therefore, the highest age susceptibility is between 3 and 6 weeks, when the bursa is at its maximum development. This age susceptibility is extended in the case of vvIBDV infection. Necropsies performed on birds that die during the acute phase (2–4 days following infection) reveal hypertrophied, hyperemic and oedematous bursas. After oral infection or inhalation, the virus

replicates primarily in the lymphocytes and macrophages of the gut-associated tissues (39).

There is a growing evidence for a role of proinflammatory cytokines in the pathogenesis of IBD. Indeed, during the acute phase of IBD, there is a dramatic infiltration of T cells around the site of virus replication, including the bursa of Fabricius, spleen and caecal tonsils. T lymphocytes do not support viral replication but are activated and exhibit up regulation of cytokine genes that has an effect on macrophage function with an exacerbated production of promediators such as interferon (IFN) γ , tumour necrosis factor (TNF) α , interleukin (IL) 6 or IL8. This cytokine storm induces a shock in the bird, which becomes prostrated and reluctant to move. A direct activation of bursal macrophages by virulent IBDV has also been demonstrated recently. Recovery from disease or subclinical infection is followed by immunosuppression with more serious consequences if the strain is very virulent and infection occurs early in life. In field conditions, chickens tend to become infected toward the age of 2–3 weeks, when MDA declines and there is considerable evidence that the virus can have an immunosuppressive effect up to the age of 6 weeks at least. Although the immunosuppression caused by IBDV is principally directed towards B lymphocytes, an effect on cell-mediated immunity has also been demonstrated, thus increasing the impact of IBDV on the immunocompetence of the chicken.

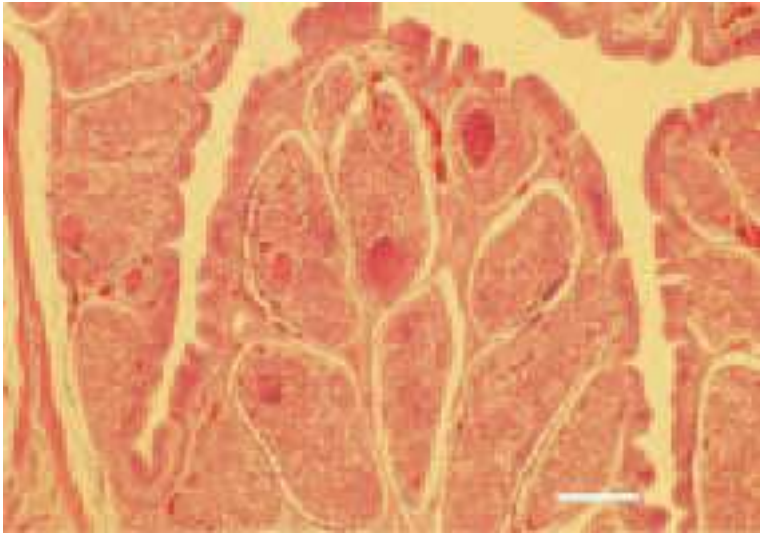
Morbidity and mortality

Infectious bursal disease is extremely contagious. In infected flocks, morbidity is high, with up to 100%, after infection, whilst mortality is variable. Until 1987, the field strains isolated was of low virulence and caused only 1% to 2% of specific mortality. However, since 1987 an increase in specific mortality has been described in different parts of the world. In the USA, new strains responsible for up to 5% of specific mortality were described. At the same time, in Europe and subsequently in Japan, high mortality rates of 50% to 60% in laying hens and 25% to 30% in broilers were observed. These hyper virulent field strains caused up to 100% mortality in specific-pathogen-free (SPF) chickens (20).

Histopathology

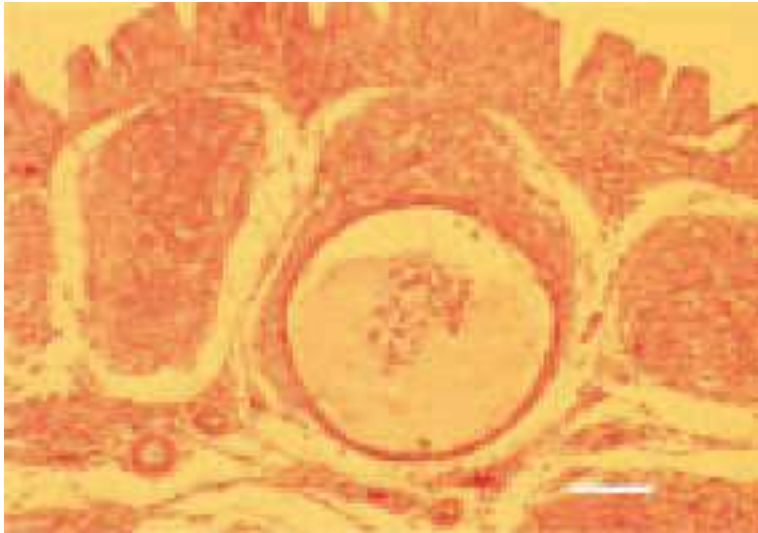
Histopathological changes occur in the bursa, spleen, Thymus, Harderian gland and cecal tonsil. The first obvious lesion occurs in the bursa of fabricus

Which was the most severely affected organ. Degeneration and necrosis of individual lymphocytes in the medullary region of the bursa occur as early as 1 day pi as showed in (picture, 5) .



Picture, 5 Marked Interfollicular Inflammatory Oedema, Haemorrhages And Inflammatory Necrotic Lesions In The Medullary Zone Of Bursal Follicles. H/E.

Lymphocyte degeneration is accompanied by nuclear pyknosis and formation of lipid droplets in the cytoplasm (40). Degeneration lymphocytes are surrounded by macrophages. Lymphocytes are replaced by heterophils, pyknotic debris and hyperplastic reticuloendothelial cells. By 3 or 4 days pi all lymphocytes have been affected. At this point of time the bursal weight increases due to edema, hypremia, and accumulation of heterophils. As the inflammatory reaction subsides, cystic cavities appear in the medullary region of the bursal follicles (Picture, 6).



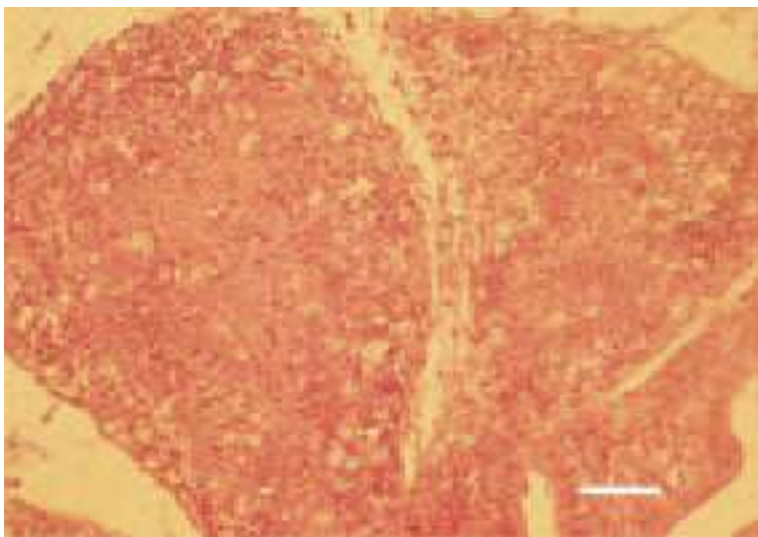
Picture, 6 Sometimes, In The Medullary zone Of follicles, cystic cavities could be formed that contain exudate, inflammatory cells and detritus mass. H/E.

Necrosis and phagocytosis of the heterophils take place and fibroplasia occurs in the interfollicular connective tissue (20). The proliferation of the bursal epithelial layer occurs producing glandular structures of columnar epithelial cells containing globules of mucin. Follicular regeneration and repopulation of follicles with the lymphocytes occur but healthy follicles are not formed during the observed time span of 18 days.

The spleen shows hyperplasia of the reticuloendothelial cells around the adenoid sheath arteries during the early stages of infection. Lymphoid necrosis occurs in the peri-arteriolar lymphoid sheath by 3 days pi. The spleen recovers shortly without any sustainable damage to the germinal follicles(20).

Changes in thymus and cecal tonsil appear shortly after infection and include areas of lymphoid necrosis and hyperplasia of the reticular and epithelial

components in the medullary region of thymic follicles, the damage is less extensive than in the bursa and is quickly repaired within 12 days pi. The harderian gland is reported to be severely affected by the virus in 1 day old chickens. Normally the gland is occupied with plasma cells than those of uninfected chickens from 1-7 weeks of age(41). However, lymphoid follicles and heterophil populations in harderian gland are not affected by IBDV infection nor could necrotic or degenerative changes be found in the acini or excretory ducts (Picture, 7).



Picture, 7 Almost Complete Disappearance Of The Normal Follicular Structure Of B. Fabricii, Resulting From Severe Degenerative Necrobiotic Lesions And Inflammatory Cell Infiltration. H/E.

In contrast, the Broiler chickens infected with IBDV at 3 weeks of age have a 51% reduction in plasma cell content at 5-14 days pi(41). Plasma cell reduction was temporary and levels became normal after 14 days.

Whereas all changes in kidney of infected chickens with IBDV appearing nonspecific and resulted from dehydration, also the liver showed slightly perivascular infiltration of monocytes (42).

General information on the immune system

The immune system is an important part of any live entity. Protection the host from infections existing in the environment such as virus, bacteria and parasites also from other non-infectious foreign substance such as protein and polysaccharide (43). Bone marrow, lymph nodes, spleen and thymus are essential elements of the immune response of chickens to microorganism. The first is the innate or (natural) immunity and the second is the adaptive (specific, acquired) immunity(42).

Innate Immunity

The innate immune system is the initial level of immune response that combats infections. Its properties are defined in the germ line. Innate immunity has no memory property. It consists of, anatomic, physiologic and phagocytic/ endocytic barriers and chemical protection such as gastric acid. These anatomic barriers are the first line of defence against invaders. They includes the skin and

mucus membrane, physiological barrier in innate response, such as PH, temperature and oxygen tension limit microbial growth. Phagocytic cells are critical in the defense against pathogens. Some primary cell of the innate immunity system include phagocytic/ endocytic barriers such as (heterophils, Monocytes, and phagocytic macrophages. These cells have specific receptors associated with common bacterial molecules. Monocytes and lymphocytes can create and secrete cytokines which are nonimmunoglobulin polypeptides in response to interaction with a specific antigen

Adaptive Immunity

Adaptive immunity is the next line of defense if the innate immunity cannot destroy the pathogen. Acquired immunity is very specific and has an immunogenic memory. The immunological memory allows this specific immunity to remember the molecular features of a pathogen that has been previously encountered and handled. Adaptive immunity includes both humoral and cell-mediated immune response (44).

Humoral Immunity

Humoral immunity can combat certain infections through circulating antibodies such as immunoglobulin (Ig) (44). The antibodies are generated as soon as germs are encountered and remain in the immune system. Immunoglobulin molecules are the cell surface receptor of B-lymphocytes derived from the bursa of Fabricius in

chicken. Antibodies in birds fall into 3 major categories, IgM, IgG(also called IgY), and IgA. It has been observed that mature B-cells which have a single antigen specificity, travel towards different lymphoid organs in order to properly interact with an antigen (45).The antibodies produced are usually incapable of struggling against viruses and some type of bacteria intracellularly. However, they are powerful at destroying extracellular pathogens.

Cell Mediated Immunity

Cell mediated immune response become active when the humoral immune response is not capable of eliminating the antigen. T-lymphocytes play an important role in the cell- mediated immunity.

T-cell can recognize antigens through the T-cell receptor (TCR) and other accessory adhesion molecules. All T-cells express the CD3 complex but T-cell has discrete subpopulation, thus distinguishing them as cytotoxic or regulatory T-Cells. Cytotoxic cells eliminate mostly virus- infected and tumor cell, they are tending to express the CD8 complex, a specific molecule on their surface (46). Regulatory T-cell, also called T-helper cells (Th) express the CD4 cell surface molecules and play a major role in the immune system. Such cells produce cytokines that are needed for T and B cells to become active. These cytokinase are capable of activating component of non-specific immunity and thus enhance better functioning of the immune system. The Th cells are suddivided into type-1 T-helper cells (Th1) and type-2 T-helper cells (Th2). The

classification of regulatory T-cells is based on the profile of cytokines produce and their function. Th1-cells an important role in cell mediated immune response while Th2-cells participate in the stimulation strong humoral immune response.

Relationship between B- and T-Cells

B-cells do not need antigen-presenting cells, because B-cells can bind directly with antigen. However, they do need cytokines created by Th cells in order to be completely active and become antibody-producing plasma cells (T dependent response). Consequently B-cells obtain support from Th-cells.

Effect of IBDV on innate immunity

IBDV modulates macrophage functions. There is indirect evidence that the in vitro phagocytic activity of these cells may be compromised. Macrophages are important cells in the immune system and the altered functions of these cells may influence normal immune responsiveness in birds.

Effect of IBDV on Humoral Immunity

IBDV has an affinity for the immature B lymphocytes and actively dividing B lymphocytes thereby causing a complete lysis of IgM bearing B cells which in turn result in decreased in circulating IgM cells. Infected chick produces less level of antibodies against the antigen (47). Chickens infected with IBDV at 1 day of age were found to be completely deficient in serum IgG and produced

only monomeric immunoglobulin M (IgM). IgG levels varied depending on the age at the time of infection. The number of B cells in peripheral blood was reduced after infection with IBDV, but T cells were not appreciably affected. The adverse effect on antibody responses is due to damage to the B cells in the bursa and the blood since the virus has a predilection for actively dividing B cells as compared to the mature B cells (47).

Effect of IBDV on cellular immunity

T-cells in spleen and peripheral circulation are affected during IBDV infection (47). The mitogenic inhibition of T-cells occurred early, during the first 3 to 5 days of virus exposure, but later returned to normal levels. During the period of mitogenic inhibition, T cells of IBDV infected chickens also failed to secrete IL-2 upon in vitro stimulation with mitogens (47).

Methods of prevention and control

The very high resistance of IBDV to physical and chemical agents accounts for persistence of the virus in the outside environment, particularly on contaminated farms, despite the farmers used most procedure for disinfection. Eradication in the affected countries therefore seems unrealistic. Prevention of IBD necessitates hygiene measures and medical prophylaxis. No vaccine can solve the problem if major sanitary precautions are not taken. These precautions include 'all-in/all-out' farming methods, cleaning and disinfection of building and observance of a 'down time' (a period of rest between depopulation and

restocking). Given the very contagious nature of the disease and the resistance of the virus, certain essential steps in the cleaning/disinfection process should be adhered to. Prior to cleaning, all insects and pests (e.g. rats and mice) must be eliminated as soon as the farm premises are empty. Old bedding and dung must be eliminated and composted. All farm equipment must be disassembled and stored in cleaning rooms located outside the farm buildings.

The buildings, immediate surroundings and farm equipment must be dry-cleaned first, in order to eliminate all dust, and then washed using hot water (60°C) with a detergent, at a pressure of 80 bar to 150 bar. A second disinfection of the full premises must be performed before the introduction of the chicks.

Feed silos must be emptied completely and cleaned inside and outside. Under no circumstances may feed remains from previous flocks be reused. Disinfection is to be undertaken only after all the buildings have been cleaned. All disinfectants are more active at a temperature above 20°C; however, chlorinated and iodinated disinfectants cannot be heated above 43°C.

The quantity of disinfectant solution to be used is approximately 4 litres per 15 m² (49).

Vaccination

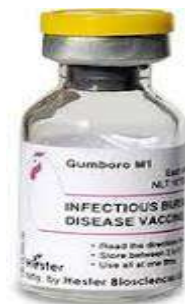
IBDV is highly infectious, very resistant in the environment and can persist in the poultry houses after cleaning and disinfection. The virus also resistant to ether and chloroform, it is inactivated at PH 12 but unaffected at PH 2. Consequently the virus can persist in the chickenhouses for long periods. Therefore, hygienic measures alone are not enough to control this disease and vaccination is the principle method used for control of IBD in chicken(50). The most common strategy followed to control IBD is by achieving passive and/or active immunity in chickens. Passive immunity is referred to transfer of IBDV specific, neutralizing antibodies from hyperimmunized parent flocks to their progeny(48). These maternally derived antibodies protect baby chick from early immunosuppressive effect caused by IBDV. Passive immunity conferred to progeny chicks normally last up to 21 days of age approximately. However, the vaccination of parent breeders with an inactivated IBDV oil-emulsified vaccine extends to range of maternal antibody protection up to 30-38 days of age. Attempts have been made to confer passive protection by performing parenteral inoculation of IBDV specific immunoglobulins in chicks of 1 day of age(48).

Active immunity is accomplished when doing vaccination of broiler breeder and layer flocks with live and/or inactivated oil-emulsified vaccines. Generally, live vaccines are used to prime the immune system so that an IBDV specific

antibody response is induced. In contrast, killed vaccines are used to boost the active immunity developed in chicken(34).

Live virus vaccine

Live virus vaccine are generally derived from the serial passage in embrionated eggs or tissue culture(34). The degree of attenuation of the vacciune strain can be classified as mild, intermediate, and hot, depending on the its ability to cause varing degree of histological lesions. Although serotype I vaccine strains cause no mortality, it is still causing different degree of bursal lesions that range from mild to moderate or even severe (34).The higher the virulency of the vaccine virus strain, the more damage that is observed in the bursa of vaccinated chickens.



The mild strai is mainly used in the breeder vaccination programme. Vaccination with the mild strain is usually affected by maternal antibody interference, therefor, such vaccine is usually used between the fourth and eight week of age,depending on weather the grandparent bird have or have not vaccinated with oil-emulsion inactivated vaccine before lay (34). Intermediate

vaccines are used for broiler and pullet vaccination and sometimes given to breeder chick when flock are at risk of early challenge of highly pathogenic strains. Day-old vaccination using intermediate vaccine may protect the chick that have insufficient maternal antibody(34). In high risks farms, two vaccination generally practice. The time of vaccination depends on the flocks maternal antibody titer. Route of vaccination is usually through drinking water, although nebulisation could also be used(34). To achieve higher maternal antibody in the progeny, vaccination of the broiler breeders with live IBD vaccine is common. Meanwhile vaccination of parent chickens with commercial live IBD vaccine under field conditions at varing age and by different routes may result in the viriable susceptibility to the disease in their chickens. One of the major problems in the use of live IBD vaccine is optimizing the time of immunizing chicken flocks. Timing of these vaccines depends apone the level for maternal antibody circulatin in serum as determined by ELISA, the rout of vaccine administration, and the pathogenecity of the vaccine virus to be used. The myriad of antibody titer with parent flocks induce a wide variability of antibody level in progeny. In consequence, some chick may be refractory to vaccination for up to 4 weeks of age, while other may be susceptible to IBDV and redy to immunizeed within the first week of age. In ova vaccination with antibody-mixed live vaccine provides an alternative mean of vaccination, in which the interference from maternal antibodies is avoided and the chickens are protectagainst IBDV, Whitfill et al., 1995 developed this type of IBD vaccine by

mixing the anti-IBDV antibody with the virus particles and this was referred as antibody-mix live vaccine. The vaccine was administered through in ovo route and was reported to be safe and more potent than the conventional IBD vaccine because it delayed the appearance of bursal lesions, produced higher geometric mean antibody titers against IBDV. The working of antibody-mixed live vaccine was thought to be related to its specific cellular interaction with the follicular dendritic cells in spleen and bursa.

Both live attenuated and inactivated (killed) vaccines are available to control the disease. It is important that live vaccines be stable, with no tendency to revert to virulence on passage. To be effective, the inactivated vaccines need to have high antigen content. Live vaccines are used to produce an active immunity in young chickens. An alternative to this is to provide chickens with passive protection by vaccinating the parents using a combination of live and killed vaccines. Effective vaccination of breeding stock is of the greatest importance

Attenuated strains of IBD viruses are used. These are referred to as either mild, intermediate, or 'intermediate plus' ('hot') vaccines.

The mild vaccines cause no bursal damage, while the intermediate vaccines cause some lymphocytic depletion in the bursa of Fabricius. None of the vaccine types results in immunosuppression when used in birds over 14 days old.

Mild vaccines are rarely used in broilers, but are used widely to prime broiler parents prior to inoculation with inactivated vaccine. Intermediate and 'hot' vaccines are more capable of overcoming very low levels of maternally derived antibodies (MDA). They may be administered by intramuscular injection, spray or in the drinking water. In the absence of MDA, the vaccines are given at 1-day old. When 1-day-old maternal antibodies are present, vaccination should be delayed until MDA in most of the flock has waned. The best schedule can be determined by serological testing of the birds to detect the time at which MDA has fallen to low level.

Inactivated or Killed Vaccines

These are essentially used to produce high, uniform levels of antibody in parent chickens so that the progeny will have high and uniform levels of MDA. The killed vaccines are manufactured in oil emulsion and given by injection. These vaccines are administered by the subcutaneous or intramuscular route at the age of sixteen to twenty weeks. They must be used in birds already sensitized by primary exposure, either to live vaccine or to field virus. This can be checked serologically. High levels of MDA can be obtained in breeder birds by giving, for example, live vaccine at approximately 8 weeks of age, followed by inactivated vaccine at approximately 18 weeks of age. Inactivated vaccines are usually used in the breeder hens for them to pass down high, uniform, and persistent antibody titeres to progeny(51).

Progeny of hens that have been vaccinated in this way have protective antibodies until the age of approximately thirty days (52).

The chicks are thus protected during the period of susceptibility to the IBDV strains that only provoke immunosuppression. However, the chicks are not protected from other highly pathogenic strains that may inflict high mortality rates at later stages (52).



The decision to use an inactivated vaccine will thus depend on the epidemiological context, namely: presence or absence of highly pathogenic strains requiring vaccination of broilers with live virus vaccines. Where no risk of infection with vvIBDVs exists, boosting of laying hens with an inactivated vaccine just before lay is fully justified (52, 53).

Vaccines use in this method is obtained either from bursal homogenates of infected chicks, or from viral cultures on embryonated eggs or tissue culture, which are then inactivated by heat which generally is ineffective due to proteindenaturation that affect the immunogenicity. Chemical inactivation with

formaldehyde and some alkylating compound like binaryethylenimine (BEI) and betapropiolactone has had success (53).

The production of antibodies [Ab] in laboratory animals is a tool used in many fields of biomedical research. Antibodies are routinely made to proteins, carbohydrates, complex lipids, and nucleic acids isolated from natural sources. In addition, modern biochemical, biosynthetic, and recombinant DNA techniques have created increasingly pure antigens [Ag]. Many of these newer antigens are small or generally weak immunogens. The small polypeptides (<10 kDa) and nonprotein antigens usually need to be conjugated to a large immunogenic carrier protein to become good immunogens. These as well as most other protein antigens (especially when administered in small quantity) need to be administered with an adjuvant to assure a high quality/high quantity, memory-enhanced antibody response by the laboratory animal. In the past few years a number of new adjuvants have become available for use in laboratory animals, although Freund's adjuvants continue to be the most commonly used despite their potential hazards.

Various adjuvant have been used in order to enhance the immune response against specific antigens since 1925, when Ramon (1925) reportef that it was possible to enhance artificially the diphteric and titanic antitoxin level by the addition of some substances. Most vaccine adjuvant use for poultry includes classical formulations, such as water-in-oil (W/O), oil- in-water (O/W), saponins and alum-based formulations. The exact mechanisms of such vaccines

that make use of a W/O emulsion as adjuvant are usually prepared by emulsifying an aqueous solution comprising the inactivated antigen. One of the most widely used adjuvant is the W/O emulsion, Freund's complete adjuvant and incomplete adjuvant. Incomplete adjuvant antigen suspended in W/O emulsions with killed *Mycobacterium tuberculosis* bacteria do stimulate strong T cell response. Freund's incomplete adjuvant has the same oil surfactant mixture as FCA but does not contain Mycobacterium(54).

Conclusion

Infectious bursal disease virus presents a certain number of characteristics that are of importance in the diagnosis and control of IBD. The disease is caused by a small, non-enveloped virus, highly resistant to the outside environment and also it is difficult to eradicate the effect of the virus in poultry farms. Substantial economic losses result from both the clinical and subclinical (or immunosuppressive) forms of the disease, but also from interactions between IBDV and other viruses. Infectious bursal disease virus has a high mutation rate and may thus give rise to viruses of modified antigenicity or increased virulence due to resistance of the causative agent to most disinfectant that use in poultry farms. Although satisfactory protection may be provided by the induction of high neutralizing antibody titres, interference of parental antibodies in vaccination has become the most important obstacle in the establishment of control programmes

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