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A REVIEW OF DIVERSITY OF INFECTIOUS BURSAL DISEASE VIRUS STRAINS IN CHICKENS

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A REVIEW OF DIVERSITY IN INFECTIOUS BURSAL DISEASE VIRUS STRAINS IN CHICKENS

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

BF	Bursa of Fabricius
caIBDV	Classical infectious bursal disease virus
dsRNA	Double stranded ribonucleic acid
hVP	Hypervariable region
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
nVarIBDV	Novel variant infectious bursal disease virus
PCR	Polymerase chain reaction
RT-PCR	Real-time polymerase chain reaction
SPF	Specific-pathogen-free
VarIBDV	Antigenic variant infectious bursal disease virus
VP	Viral protein
vvIBDV	Very virulent infectious bursal disease virus

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterianr untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir.

ULASAN KEPELBAGAIAN STRAIN VIRUS PENYAKIT BURSAL BERJANGKIT DALAM AYAM

Oleh

Chaveewan A/P Bani

2021

Penyelia: Professor Dato' Dr Mohd Hair Bejo

Penyelia Bersama: Dr Mazlina binti Mazlan

Virus penyakit bursal berjangkit (IBD) ialah etiologi penyakit kekurangan daya tahan ayam yang sangat menular dikenali sebagai penyakit Gumboro yang menyebabkan kerugian ekonomi yang teruk kepada industri ayam di seluruh dunia. Virus IBD (IBDV) ialah Birnavirus dengan genom RNA dua rantai dua bahagian. Segmen virus ditetapkan sebagai A dan B. IBDV dikelaskan kepada tiga strain berdasarkan genotip dan patotip iaitu klasik, varian dan sangat ganas atau tujuh kumpulan-gen berasaskan genotip (Kumpulan-gen 1 - 7). Objektif kajian ini adalah untuk mengkaji kepelbagaian strain IBDV dalam ayam. Persamaan dan perbezaan tiga strain IBDV dibandingkan berdasarkan patotaip dan genotip. Klasifikasi IBDV kepada tujuh kumpulan-gen berdasarkan genotip telah dikaji semula. Kaedah direka berdasarkan garis panduan PRISMA. Data yang diterbitkan antara 1962 hingga 2021 dianalisis berdasarkan negara, tanda klinikal, lesi kasar dan histologi serta ciri molekul IBDV. Daripada 287 kajian pada mulanya mendapati hanya tiga puluh lapan memenuhi kriteria kemasukan. Virus ini menjangkiti bursa Fabricius yang memusnahkan limfosit B, mengakibatkan morbiditi, kematian dan imunosupresi. IBDV yang sangat ganas

menyebabkan kematian yang lebih tinggi daripada strain klasik, varian dan novel. Selain itu, imunosupresi meningkatkan kerentanan ayam kepada jangkitan lain dan mengganggu vaksinasi terhadap penyakit itu, hasil daripada evolusi strain baru. Tujuh kumpulan-gen berdasarkan genotaip (Kumpulan-gen 1 hingga 7). Disimpulkan bahawa evolusi IBDV di lapangan disebabkan oleh strain medan yang beredar dan vaksinasi terurai, mengakibatkan morbiditi, kematian dan imunosupresi, membenarkan keperluan untuk vaksin pelindung yang lebih selamat, dan pelaksanaan langkah biosekuriti yang ketat untuk meminimumkan kerugian kepada industri ayam.

Kata kunci: Virus penyakit bursa berjangkit (IBDV), klasik, varian, sangat ganas, kumpulan-gen

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

A REVIEW OF DIVERSITY OF INFECTIOUS BURSAL DISEASE VIRUS STRAINS IN CHICKENS

By

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2021

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Co-supervisor: Dr Mazlina binti Mazlan

Infectious bursal disease (IBD) virus is the aetiological agent of a highly contagious chicken immunodeficiency disorder known as Gumboro disease, which cause severe economic loses to the poultry industry worldwide. IBD virus (IBDV) is a Birnavirus with a bisegmented, double-stranded RNA (dsRNA) genome. The virus segments designated as A and B. IBDVs were classified into three strains based on genotype and pathotype namely classical, variant and very virulent or seven genogroups based genotype (Genogroup 1 - 7). The objective of this study was to review on the diversity of IBDV strains in chickens. The similarities and differences of three strains of IBDVs were compared based on pathotype and genotype. The classification of IBDV into seven genogroups based on genotype was reviewed. Methods were designed based on the PRISMA guideline. Data published between 1962 to 2021 and data were analysed based on country, clinical signs, gross and histological lesion as well as molecular characteristics of IBDV. From 287 studies initially found only thirty-eight met the inclusion criteria. The virus infects the bursa of Fabricius which destroy B lymphocytes, resulting in morbidity, mortality and immunosuppression. Very virulent

IBDV cause higher mortality than classical, variant and novel variant strains. Moreover, immunosuppression enhances the susceptibility of chickens to other infections and interferes with vaccination against the disease, resulted from evolution of new strains. Seven genogroups based on genotype including Genogroups 1 to 7. It was concluded that the evolution of the IBDVs in the field due to circulating field strains and vaccination break down, resulting into morbidity, mortality and immunosuppression, justifying the need for safer protective vaccines, and implementation of strict biosecurity measures to minimizes loss to poultry industry.

Keywords

Infectious bursal disease virus (IBDV), classical, variant, very virulent, genogroup

1.0 INTRODUCTION

Infectious bursal disease (IBD), also known as Gumboro disease, an immunosuppressive disease of poultry leads to high morbidity and mortality, and also heavy economical losses to poultry industry (van den Berg *et al.*, 2000; Michel *et al,* 2017). It was first reported in southern Delware, Gumboro in 1957 (Cosgrove, 1962). IBD virus (IBDV) is highly resistance to chemical and physical agent that allow them to constantly persist in the environment (Parkhurst,1964), thus the economic impact of the disease is influenced by pathogenicity of the virus, susceptibility of the flock, presence of other prevalent pathogens, the environment and poor management practices (Eterradossi & Saif, 2013).

The causative agent for IBD is bisegmented double stranded RNA virus with segments A and B, that belongs to the family Birnaviridae of the genus Avibirnavirus (Muller *et al.,* 1979). IBDV has two serotypes: pathogenic strains are classified as serotype 1 viruses, while serotype 2 strains are obtained from turkeys and are avirulent to chickens (Mahgoub, 2012; Qin & Zheng 2017). IBD is highly contagious disease in young chickens aged between 3 to 6 weeks are most likely to be affected, and is characterised by the destruction of the lymphoid organs, especially bursa of Fabricius, where B lymphocytes undergo maturation and differentiation. Acutely infected chickens will normally develop clinical signs such as fatigue, prostration, dehydrated, watery diarrhea, ruffled feathers and death (van den Berg *et al.*, 2000).

In 1957, Delware, Gumboro had the first outbreak of classical IBDV (calBDV) (Cosgrove, 1962). It was characterised by flock morbidity ranging from 10% to 25% and mortality ranging from 10% to 50% (Eterradossi & Saif, 2013). Variant IBDV (vaIBDV) was initially identified in the USA, China, and Australia in 1983. Classical IBDV vaccination did not provide protection against these new 'variant strains' of the disease in chickens, which were overwhelmed by an immunosuppressive form of the

disease (Lasher & Shane, 1994). The very virulent IBDV (vvIBDV) strains, a newly developed strain related to high mortality, were initially discovered in Europe in the late 1980s, and IBD was thought to be limited to the virus's immunosuppressive effect (Lasher & Shane, 1994). The vvIBDV had caused high acute mortality as well as caused severe economic losses worldwide (Escaffre *et al.,* 2013). Epidemics of new variant IBDV (nVarIBDV) have recently been reported in China, followed by Japan, Korea, and Malaysia (Fan *et al.,* 2019; Thai *et al.,* 2021). Genotype A2dB1 was designated to the nVarIBDV strains, which are genetically distinct from the early variation IBDV that was first identified in America (Wang *et al.,* 2021a; Wang *et al.,* 2021b). The virus's antigenicity and virulence are constantly changing in the field, resulting in huge economic losses and severe mortality in chickens (Jackwood *et al.,* 2011), rendering vaccines and vaccine protocols less effective (Jackwood *et al.,* 2008).

IBDV has recently been divided into seven genogroups based on significant differences in amino acids in the hypervariable region of the capsid protein VP2 (hVP2) between the groups. Genogroup 1 consists primarily of the classical IBDV (caIBDV) and were discovered across the world; genogroup 2 consists of the antigenic variant IBDV (vaIBDV), which is prevalent in America; and genogroup 3 consists primarily of the very virulent IBDV (vvIBDV) pathotype and vvIBDV reassortants, which are distributed worldwide (Michel & Jackwood, 2017). Viral isolates that did not specifically fall into one of the three major genogroups were classified separately. Using the genogroup classification approach, these viruses were divided into four new genogroups 4 – 7. A sample of genogroup 4 from the United Arab Emirates. Recombinant classical and variant of Genogroup 5 virus strains from Mexico (Jackwood, 2012). Genogroup 6 is prevalent in non-Asian countries, particularly in Asia's upper regions and the Middle East (Lupini *et al.,* 2016).

Genogroup 7 was made up of viruses from Australia comprises two distinct groups of IBDV, the classical strains and antigenic variant strains (Michel & Jackwood, 2017).

The objective of this study was to review on the diversity of IBDV strains in chickens. The similarities and differences of three strains of IBDVs were compared based on pathotype and genotype. The classification of IBDV into seven genogroups based on genotype was reviewed.

2.0 MATERIALS AND METHODS

2.1 *Data collection and inclusion criteria*

The study was conducted to systematically review scientific journal articles on infectious bursal disease virus in English published from 1962 to 2021. The study followed the PRISMA statement (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses-2010*). The articles were obtained from PubMed (https://pubmed.ncbi.nlm.nih.gov/), ScienceDirect (https://www.sciencedirect.com/) and Scopus (https://www.scopus.com/home.uri) databases. For the systematization of information, a database was built that included the references of all selected publications, as well as the title, author, year of publication, country where the study was conducted, and publication language. From the results, three strains of IBDV and seven genogroups were identified and reviewed.

2.2 *Exclusion criteria*

The case reports in other species such as turkeys, pigeon and guinea fowl, and case reports concurrent with other disease such as Chicken Infectious Anemia, Newcastle disease and Marek's disease studies were excluded. Unrelated to classification of IBDV studies were also excluded.

2.3 Search strategy

The processes for searching, selecting, and collecting the articles in the databases were conducted using keywords combined with Boolean operators (Table 1). The "Limit-to" function included in the search engine was used to define all open access and document type.

Table 1: Query string inserted in the database

Keywords	Query string
Infectious Bursal Disease Virus, Classical IBDV, Variant IBDV, Very virulent IBDV, Novel variant IBDV	("infectious bursal disease virus OR ibdv") AND ("classical" OR caibdv) AND (LIMIT-TO (OA, "all")) AND (LIMIT-TO (DOCTYPE "re")) ("infectious bursal disease virus OR ibdv") AND ("antigenic variant" OR "variant" OR "avibdv") AND (LIMIT-TO (OA, "all")) ("infectious bursal disease virus OR ibdv") AND ("very virulent" OR "vvibdv" OR "genogroup3") AND (LIMIT-TO (OA, "all")) ("infectious bursal disease virus OR ibdv") AND ("novel variant" OR "naribdv") AND (LIMIT-TO (OA, "all"))
Infectious Bursal Disease Virus, Genogroup, Genotype	("infectious bursal disease virus OR ibdv") AND ("genogroup") AND (LIMIT-TO (OA, "all")) AND (LIMIT-TO (DOCTYPE "ar")) ("infectious bursal disease virus OR ibdv") AND ("genotype") AND (LIMIT-TO (OA, "all")) AND (LIMIT-TO (DOCTYPE "ar"))

2.4 *PRISMA diagram*

Summary of the literature search is as shown in Figure 1.

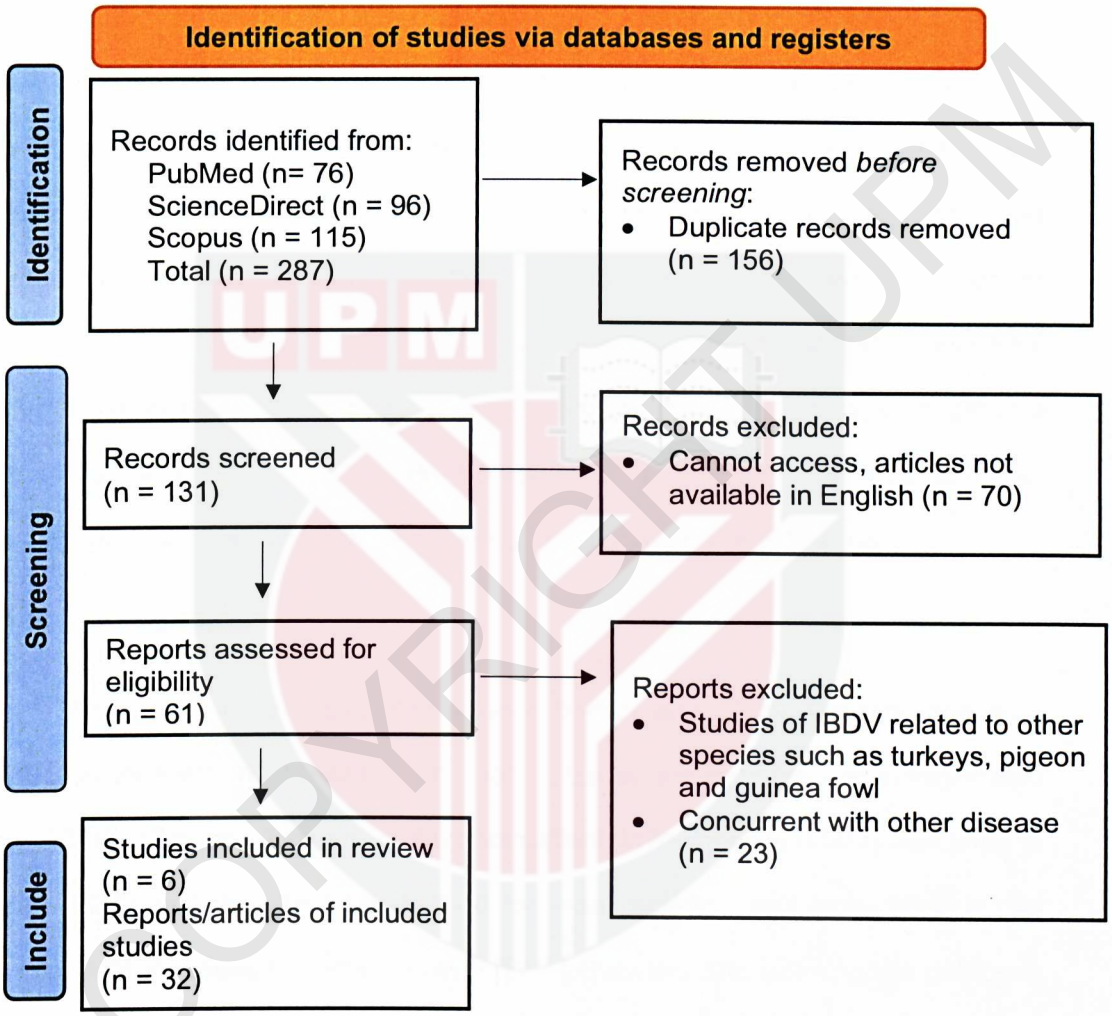

Figure 1: Summary of the literature search

3.0 RESULTS

3.1 *Source and collection of samples*

Based on Michel & Jackwood (2017), 90 samples from Algeria, Colombia, Ecuador, Egypt, Fiji, France, Guatemala, India, Indonesia, Iraq, Jordan, Kazakhstan, the Kingdom of Saudi Arabia, Kuwait, Malaysia, Mexico, Morocco, Philippines, Russia, the United Arab Emirates, the United Kingdom, the United States, and Vietnam were collected and examined for suspected IBDV, based on marked changes in the amino acids in the hypervariable region of the capsid protein VP2 (hvVP2). Genogroup 1 has 15 samples, Genogroup 2 has 27 samples, Genogroup 3 has 41 samples, Genogroup 4 has one sample, Genogroup 5 has three samples, Genogroup 6 has two samples, and Genogroup 7 has only one sample.

3.2 *Virus isolation*

The bursa of infected chickens was collected, and a 20% bursal homogenate in PBS solution was prepared from the pooled bursa. Infecting chicken embryonated eggs (CEE) after inoculation onto the chorioallantoic membrane (CAM) was used to isolate IBDV strains. The best method for virus isolation was inoculating bursal homogenate into specific-pathogen-free (SPF) chickens and isolating the pathogen from the bursal tissues three days later. The virus was titrated in 10-day-old chicken embryos by the CAM route, and endpoint titers were determined based on particular fatalities. After 3 to 5 days post CEE inoculation, the samples with IBDV strains were found to cause up to 100% mortality, hemorrhages and edema found on the skin of dead embryos with congestion and thickening on the CAM (van den Berg *et al.,* 2000).

3.3 *Molecular diagnostic tests*

Sequencing the hVP2 gene and combining it with pathogenicity tests in chickens has been the most reliable and commonly accepted method for detecting

IBDV strains. Amplified of hvVP2 by reverse transcription polymerase chain reaction (RT-PCR), followed by nucleotide sequencing and phylogenetic analysis, is the most useful tool for classifying IBDV strains. Based on 23 countries, 90 samples were collected across Europe, North and South America, and Asia were sequenced for IBDV. These samples were assigned to certain genogroups based on molecular characteristics (Michel & Jackwood, 2017).

3.4 *Reverse transcription polymerase chain reaction (RT-PCR)*

The hypervariable region of the IBDV VP2 gene was identified and amplified using RT-PCR. The RT-PCR was carried out using the AgPath-IDTM One-Step RT-PCR Reagents Kit. A 579-bp fragment of the VP2 hypervariable region (hvVP2) was amplified using the 743-F (5-GCCCAGAGTCTACACCAT-3) and 1331-R (5-ATGGCTCCTGTCAAATCG-3) primers (Michel & Jackwood, 2017; Jackwood *et al.,* 2018). According to Michel & Jackwood (2017), to determine the phylogenetic tree of IBDV hvVP2, the researcher conducted RT-PCR followed by nucleotide sequencing. A Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI) was conducted to fixed the RT-PCR products for sequencing. DNA sequences were submitted to GenBank as well as nucleotide and amino acid sequences were aligned with the reference strains from GenBank using Geneious® 8.1.8.

3.5 *Clinical signs*

The severity of clinical symptoms and immunosuppression associated with IBDV infection are related to the chickens' immune state, age and genetic background, and the virulence of the infecting virus strain (Wagari, 2021). Chickens infected between 3 and 6 weeks of ages have the most severe IBD manifestations. Clinical signs in chicks less than 2 weeks old and chickens older than 6 weeks of age are uncommon. Excretion of the virus might begin as soon as 24 hours after infection.

In most cases, mortality peaks and then declines over a period of 5 to 7 days. Depression, trembling, white watery diarrhoea, ruffled feathers, acute prostration, vent picking, vent feathers stained with urates, anorexia, dehydration, increased water consumption, and death have all been reported as symptoms of the disease (Escaffre *et al.,* 2013; Eterradossi & Saif, 2013).

Clinical signs of infection with calBDV strains were whitish or watery diarrhoea, vent feathers soiled by urine material, dehydration, anorexia, depression, tremor, acute prostration, and death (Cosgrove, 1962), with a high fatality rate (10% to 50%) (Eterradossi & Saif, 2013). The studies of susceptible chickens exposed to vaIBDV reported to have low mortality rate and considered subclinical (Eterradossi & Saif, 2013; Thai *et al.,* 2020). Susceptible chickens exposed to vvIBDV were reported with clinical signs of severe depression, ruffled feathers, severe prostration, diarrhea and dehydration (Escaffre *et al.,* 2013). The clinical signs of vvIBDV were reported the most severe clinical signs with very high mortality rate (50% to 100%) (Eterradossi & Saif, 2013). nVarIBDV were reported subclincal with low or no mortality (Fan *et al.,* 2020).

3.6 *Gross lesions*

Despite the fact that IBD affects a variety of lymphoid organs, the virus's primary target is the bursa of Fabricius, which serves as a reservoir for B lymphocytes in chickens. In 3 to 4 days after infection, post-mortem examination revealed hypertrophy, oedematous, and hemorrhagic bursa in the infected chickens. The bursa may recover to normal size by the fifth day. Darkened pectoral muscles and many petechial haemorrhage masses in the thigh and pectoral muscles were common in affected chickens. A swollen spleen may also be present (Eterradossi & Saif, 2013).

Infected chickens with calBDV strains, ecchymotic hemorrhages were observed in the enlarged bursa of Fabricius and pale yellow bursal colour with yellowish transudate of bursa (Eterradossi & Saif, 2013). Meanwhile, haemorrhages were observed in the breast and thigh muscles in some of the affected chickens (Pikula *et al.,* 2018). The studies of valBDV of necropsy reported in susceptible chickens with severe bursa atrophy (Eterradossi & Saif, 2013; Thai *et al.,* 2020). The studies reported on gross lesions on the mucosal and serosal surfaces of samples from infected chickens with vvIBDV included enlarged bursas with varied degrees of petechial haemorrhages (Figure, 2) (Mato *et al.,* 2020; Stoute *et al.,* 2019). In addition, at day 3 post infection, haemorrhagic bursa, haemorrhages at the juncture of the proventriculus and ventriculus of gizzard, decrease in thymic weight as well as more severe lesions in cecal tonsils, thymus, spleen and bone marrow were observed in very virulent strains. At day 10 post infection, the bursa of Fabricius exhibited severe atrophy (Etarradossi & Saif, 2013). Gross lesion observed in susceptible chickens exposed to nVarIBDV is bursa of Fabricius atrophy, haemorrhages, yellowish with inflammatory exudation as well as spleen atrophy (Fan *et al.,* 2019).

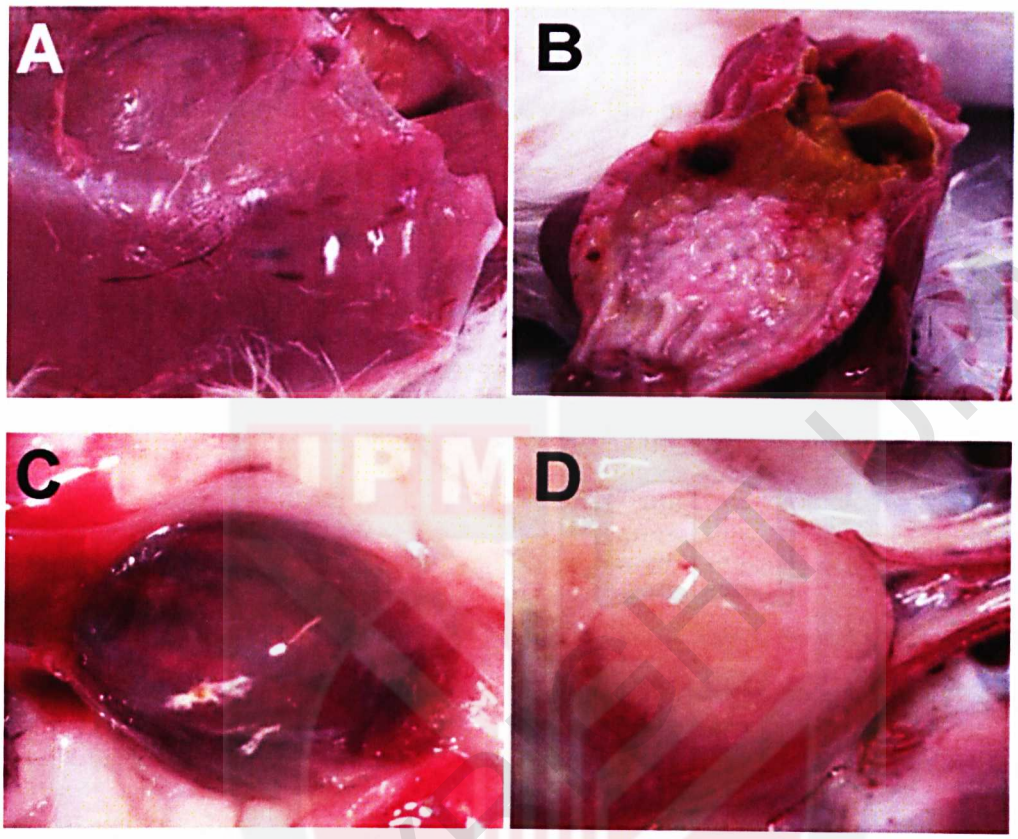

Figure 2: Gross lesions in 5 weeks old SPF chickens infected with very virulent strain (A-C) and a reassortant IBDV (D) strain at 3 days after infection. Ecchymotic haemorrhages in breast muscle (A), ecchymotic haemorrhages in the proventriculus at the junction of the gizzard (B), and bursa haemorrhages with peribursal exudate in vvIBDV strains (C). Oedematous, yellowish bursa (D) (Mato *et al.,* 2020).

3.7 *Histological lesions*

The primary target organ of IBDV is the bursa of Fabricius which is the reservoir of B lymphocytes in chickens. As a result of the virus's stimulation, mature and competent lymphocytes will proliferate, whereas immature lymphocytes will be destroyed. Macroscopic lesions are observed principally in the bursa which presents all stages of inflammation following acute infection. Bursa of Fabricius was collected and histology preparations were conducted as previously described. Briefly, each bursa sample was analyzed and assessed individually for histological abnormalities. After 3 to 5 days post inoculation, the bursa of Fabricius was infiltrated with inflammatory cells such as heterophils and macrophages, as well as lymphocyte depletion or necrosis of the bursal follicle's medullary and cortical areas. The bursal tissue showed necrosis, atrophy, or fibrosis of lymphoid follicles on day 10 after inoculation (Mato *et al.,* 2020).

It was reported that susceptible chickens exposed to calBDV revealed depletion of B lymphocytes as well as infiltration of B cells and macrophages (Eterradossi & Saif, 2013). The studies reported histopathological analysis revealed lymphocyte degeneration and necrosis of lymphocytes in the bursa of chickens infected with valBDV as well as atrophy of follicles (Figure, 3a) (Yamazaki *et al.,* 2017; Thai *et al.,* 2020). The studies reported histologic lesion with vvIBDV had caused severe lymphocyte depletion as well as necrosis and haemorrhage in bursa of Fabricius, and severe atrophy of follicles (Figure, 3b) (Saif, 1998; Dobner *et. al.,*2019). In addition, macroscopic lesions in other lymphoid organs (thymus, spleen, caecal tonsils, Harderian glands, and Payer's patches) may be shown on the acute phase of the disease caused by very virulent strains with a high mortality rate (Stoute *et al.,*

2019). Chicken infected with nVarIBDV revealed lymphocyte depletion, macrophages infiltration, fibrous tissue proliferation and severe atrophy of follicles (Fan *et al.,* 2019).

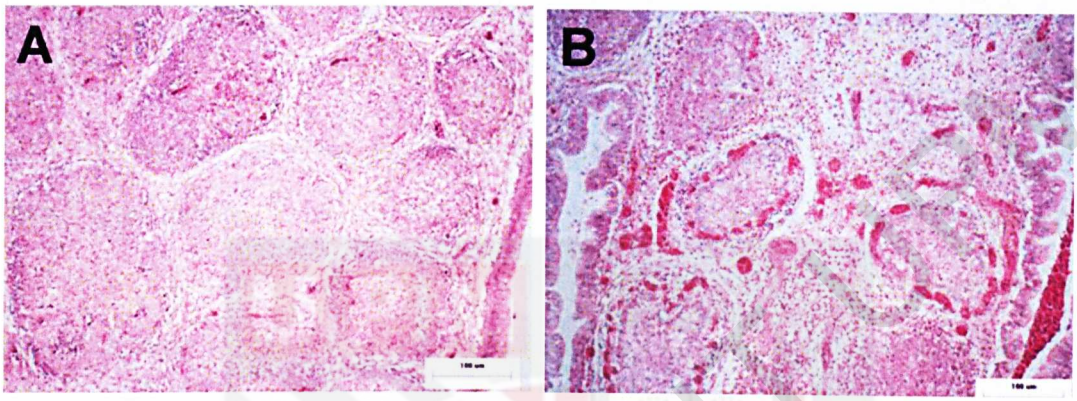

Figure 3: Lesion in valBDV (A) at 3 days post infection, lymphocyte necrosis and depletion in the follicles, and moderate inflammatory reaction (infiltration by heterophil granulocytes and macrophages, moderate oedema in the interfollicular tissues). Complete destruction of the bursal tissues due to lymphocyte necrosis and severe inflammation in vvIBDV (B) at 3 days post infection (Mato *et al.,* 2020).

3.8 *Phylogenetic analysis of hvVP2*

According to Michel & Jackwood (2017), 90 samples were divided into seven genogroups, which are relatively similar to the classification of serotypes or pathotypes (Table 2). Genogroups of IBDV were identified for segments A and B sequences respectively based on phylogenetic analysis. Based on the combination of each segments A and B genogroups, IBDV strains were classified into different genotypes. Based on phylogenetic analysis of segment A, it consists of serotype 1 and serotype 2 IBDV. Hence, serotype 1 strains can be further divided into seven genogroups (genogroups 1 - 7), whilst serotype 2 strains consist of one genogroup (genogroup 2). In serotype 1 strains, segment A were classified into seven

genogroups which are 1 (classical), 2 (variant), 3 (very virulent), 4 (distinct), 5 (variant/classical recombinant strains), 6 (atypical Italian) and 7 (early Australian). Meanwhile, the single genogroup under serotype 2 is classified as A0 (Islam *et al.,* 2021).

According to Michel & Jackwood (2017), genogroup 1 of IBDV (classical IBDV) was identified all around the world. The genetic hallmark of amino acid which at location 222, consist of more than 80% of isolates had Proline (P) and others shared (Serine or Alanine) in the variable area. Asparagine (N) was identified at position 299 in all genogroup 1 isolates (Ali Khan *et al.,* 2019).

The antigenic variation IBDV of Genogroup 2 is still widely spread in the Americas. Threonine (T) was the amino acid found in the evolutionarily conserved position 222 in genogroup 2. Based on previous study, this genogroup 2 is still conserved in the South and North America. In previous study reported this group (Genogroup 2) was confused with variant or classical recombinant strains (Genogroup 5), but they had been categorised into separate group clearly (Ali Khan *et al.,* 2019).

Genogroup 3 generally very virulent IBDV pathotype and reassortants have been identified all around the world. According to Michel & Jackwood (2017), the deduced amino acid sequence comprises the amino acid patterns A222, I242, I256, I294 and S299, which are common in genogroup 3 strains. In genogroup 3 viruses, the shift from a P to an A amino acid at position 222 in the first major hydrophilic region could result in considerable antigenic alterations (Bari, 2021). Viruses from Egypt, India, Indonesia, and Malaysia were discovered in various distinct branches (Michel & Jackwood, 2017; Ali Khan *et al.,* 2019).

Based on Michel & Jackwood (2017), a genogroup 4 sample from the United Arab Emirates (741 UAE) was studied, and it was shown to be closely linked to the distinctive IBDV that is endemic in South America. There are common amino acids at genetic markers 222S, 242V, 256V, 279N and 294L, however amino acids at position 299 displays Asparagine (N)/Serine (S)/Histidine (H) (Ali Khan *et al.,* 2019).

Genogroup 5 consist of recombination between variant or classical strains of viruses from Mexico (Michel & Jackwood, 2017). According to the studies, the PBC loop of these viruses has variation type amino acid sequences, whereas the PFG loop is more related to classical strains (Ali Khan *et al.,* 2019).

ITA strains are widespread in Italy which suggest an evolution of the virus strains in response to a selective pressure exerted by vaccines (Lupini *et al*., 2016). According to the studies, a sample of genogroup 6 from the Kingdom of Saudi Arabia (751 KSA and 772 KSA) did not show an equivalent match in GenBank, but it did show 92.26 to 93.64% similarity to the ITA genotype discovered in Italy and 94.02 to 95.40% similarity to isolate IBDVRF-5/94 from Russia (Michel & Jackwood, 2017).

Genogroup 7 consist strains of viruses from Australia and Russia (429_Russia) (Michel & Jackwood, 2017). The member of genogroup 7 have the same genetic markers at position 222 Proline (P), 242V, 256V, 279G, 294L and 299S. The studies reported genogroup A7 is restricted in non-Asian regions (Ali Khan *et al.,* 2019).

Table 2: Classification of IBDV isolates by genogroup (Michel & Jackwood, 2017)

Genogroups	Classification	Country	Reference strains (GenBank accession number)
1	Classical	Algeria, Colombia, Egypt, Fiji, France, Mexico, Morocco, Philippines, Russia, UK, United States	228E (AF457104) D78 (AF499929) F52-70 (AY321953) Lukert (AY918948) STC (D00499)
2	Antigenic variant	Ecuador, Guatemala, Mexico, United States	AL-2 (JF736011) DelE (AF133904) T1 (AF281238)
3	Very virulent	Algeria, Colombia, Egypt, Guatemala, India, Indonesia, Iraq, Jordan, Kazakhstan, Kuwait, Malaysia, Russia, United States, Vietnam	Henan (KT884486) HK46 (AF092943) OKYM (AF092943) UK661 (NC_004178)
4	Distinct	United Arab Emirates	dIBDV/UY/2014/2202 (KT336459) MG4 (JN982252) TY2 (LC136880)
5	Variant/classical recombinant	Mexico	Mexico04M101 (DQ916210)
6	ITA	Kingdom of Saudi Arabia	ITA-02 (JN852986)
7	Australian	Russia	V877-W (HM071991)

4.0 DISCUSSION

Infectious bursal disease is one of the most common infectious immunosuppressive diseases that affect poultry worldwide. For the worldwide poultry industry, studies focused on better control of this economically devastating disease is vital. Young chickens are more susceptible to a variety of invasive chicken infections that are generally non-pathogenic in healthy chickens due to the possibility of possibly irreversible immune suppression caused by IBDV (Saif, 1991). IBDV control has only been possible with effective vaccines, but vacciantion efforts have been limited by the fact that frequent IBD viral genetic mutations, reassortment of genome segments, and recombination have the potential to enhance the virulence and alter antigenicity, making vaccines and vaccine protocols less efficient (Jackwood *et al.,* 2011).

IBDV was first reported in the USA in 1957 (Cosgrove, 1962). It was first discovered in Delaware, Gumboro, the clinical signs of infected chickens with classical strain showed were whitish or watery diarrhoea, soiled vent feathers, followed by dehydration, anorexia, depression, trembling, severe prostration, and eventually death. High mortality rate in susceptible chicken with classical strain of IBDV is generally 10% to 50% (Eterradossi & Saif, 2013). Moreover, IBD generally lasts for 5 to 7 days. The classic IBDV lesion is distinguished by a pale yellow bursal colour and a yellowish bursa transudate, haemorrhages in the breast and thigh muscles, as well as enlargement of the bursa of Fabricius (Eterradossi & Saif, 2013). Genogroup 1 classified as calBDV were diversified and reported globally (Jackwood *et al.,* 2017). The genetic hallmark of amino acid in genogroup 1 is at location 222, as more than 80% of isolated had Proline (P) (Ali Khan *et al,* 2019).

Variant strains of IBDV became predominant in the America in the late of 1980s, resulted in severe bursal damage and cause immunosuppression and subclinical infection as well as vaccination failure. It was found that classical strain immunizations caused severe immunosuppression epidemics because it also does not include protective immunity against variant strains (Yamazaki et al., 2017). IBD-induced immunosuppression enhances the poultry industry's loss of profits because of decreased raising flock productivity. During post mortem examination, the rapid atrophy of bursal without prominent inflammation, is one of the typical lesions observed in variant IBDV strain in infected chickens (Thai *et al.,* 2021). In addition, a new strain has been identified in China in 2017, which is novel variant IBDV strain stated clearly belong to genogroup 2 (variant strain). Novel variant strain did not cause any obvious gross clinical lesion but it induced severe subclinical infection (Fan *et al.,* 2020).

After vvIBDV strains were first discovered in Europe in 1980's, these viruses were reported in various of countries. Various prevalence of very virulent strains of IBDV have been reported in Europe and the USA (Yilmaz *et al.,* 2019). Based on Mato *et al.,* (2020), clinical symptoms in chickens infected with the vvIBDV strain were greenish watery diarrhoea, dehydration, depression, ruffled feathers, lethargy, low feed intake, poor growth, and death starting on day 2 post-infection. The mortality can reach up to 50% to 100% with very virulent strain. Meanwhile vvIBDV had become an issue in Europe in the late of 1980s, which caused mortality rate up to 90% to 100%, susceptible in 4-week-old leghorn chickens (Eterradossi & Saif, 2013). During necropsy, the dead chickens were dehydrated, with muscular haemorrhages noticeable. A large number of viruses can be measured at day 3 post infection. Infected chickens' Fabricius bursae revealed severe enlargement, yellowish discolouration, oedema, and varying degrees of haemorrhages (Mato *et al.,* 2020).

On day 3 post infection, the Fabricius bursa starts increasing in size and weight due to oedema. On day 4, the bursa was double its normal weight, and the size of the bursa began to decrease. The bursa, on the other hand, returns to normal weight on day 5 and continues to atrophy until day 8, when it is about one-third of its previous weight or less. Furthermore, the strain with very virulent caused more severe lesions in the cecal tonsils, thymus, spleen, and bone marrow. (Eterradossi & Saif, 2013).

Michel & Jackwood (2017) reported that infected chicken with IBDV strains and genogroups (G1 – G7) showed some variation in clinical signs, gross lesions and histological lesions depending on their pathogenicity. However, the studies reported that very vvIBDV strains which also classified as Genogroup 3 have the highest mortality rate as well as severe gross lesion and histological lesion in the bursa of Fabricius compare to other IBDV strains (Stoute *et al.,* 2019). The vvIBDV strains have been reported 50% to 60% mortality in laying hens, 25% to 30% mortality in broilers, and 90% to 100% mortality in susceptible specific pathogen free (SPF) leghorns (van den Berg *et al.,* 2000; Wagari, 2021). The caIBDV have high mortality rate of 10% to 50% (Eterradossi & Saif, 2013) whereas vaIBDV had low mortality rate (Thai *et al.,* 2021). The nVarIBDV had no mortality in infected chickens (Fan *et al.,* 2019).

Stoute *et al.,* (2019) reported that bursa samples infected with IBDV strains were examined and evaluated for histological abnormalities, by observing depletion of lymphocyte in susceptible chickens with caIBDV. However, the studies had reported that vvIBDV (genogroup 3) have severe lymphocyte depletion in bursa of Fabricius, and severe atrophy of follicles (Saif, 1998). Yamazaki *et al.,* (2017) reported that vaIBDV strains showed degeneration and necrosis of lymphocytes as

well as atrophy of follicles, whereas nVarIBDV strains showed depletion of lymphocytes and severe atrophy of follicles (Fan *et al.,* 2019).

Van den Berg (2000) and Muller *et al.,* (2003) found out that isolated strains were based on the pathogenicity and the sensitive test for detecting IBDV infection in chicken flocks is RT-PCR identification of IBDV strains. According to Petkov et al., (2007), to determine the pathogenicity and virulence of collected field strains, the hypervariable region of VP2 was sequenced. IBDV strains can be divided into seven genogroups using this method (G1 - G7). According to Ali Khan et al., (2019), genogroups 1, 3, and 4 had shared genetic information globally, however other genogroups were preserved in a specific region.

Michel & Jackwood (2017) proposed a phylogenetic analysis of the hypervariable VP2 nucleotide sequence based on genogroups and a classification scheme for IBDV based on sequence data publicly accessible in GenBank. The traditional phenotypes of classical IBDV, variant IBDV, and very virulent IBDV were grouped into genogroups 1, 2, and 3 according to the new classification scheme. Other IBDVs with evolutionary traits different from the other three phenotypes were divided into genogroups 4, 5, 6, and 7 (Michel & Jackwood, 2017; Jackwood *et al.,* 2018). The distinct IBDV (dIBDV) strains were isolated from South America which formed genogroup 4. IBDV of genogroup 4 have been reported found worldwide, however, Latin America is where the virus is most usually found. Mexico IBDV strains with homologous recombination traits of variant and classical strains were found in Genogroup 5 (Jackwood, 2012). Both PDE and PHI show amino acid alterations in this genogroup. The existence of 251N and 254N in the PDE loop distinguishes it from the reference variant and classical strains. Genogroup 6 is made up of ITA-like strains that have a similar identity to the distinctive IBDV seen in Italy. They proved

to be most closely linked to viruses of the Italian ITA genotype, with which they shared almost 97% of their amino acid sequence. The studies had been hypothesized that the ITA viruses arose under selective pressure exerted by vaccines (Lupini et al., 2016). Genogroup 7 consist of Australian reference strains was isolated from Russia (429 Russia). The vaccine virus utilised and persisted in the specific flocks was determined to be 100% identical to the Australian 877 vaccination strain, indicating that the vaccine virus was used and survived in the individual flocks (Jackwood *et al.*, 2018; Islam *et al.,* 2021).

To prevent IBD, strict hygiene precautions and vacciantion with standard live attenuated and inactivated viral vaccines were applied. The virus may survive in a poultry house for 122 days and 52 days within feed and water, making it difficult to get rid of the resilient and persistent IBDV particles (Mahgoub, 2012). To control IBDV, normal hygiene precautions must be strictly maintained in the poultry farm. Disinfection can help to reduce virus concentration and thus minimize the risk of transmission. Mosquitoes, mealworms, and smaller rodents are mechanical vectors that must be eradicated to prevent spread of IBDV in poultry farm (Van den Berg *et al.,* 2000). On farms where IBDV outbreaks have occurred, the virus may be considered endemic. The IBD virus will be introduced to the young birds at a young age if the area are not cleaned and disinfect properly. Long-term immune response depletion can result from the condition, and immunocompromised chickens do not respond well to immunisation and are prone to other infections. Slaughtering diseased chickens and preventing other flocks from becoming infected is quite expensive. As a result, vaccination is still the most effective way to reduce IBD in chicken farms. Failure of vacciantion do happen, nevertheless, due to the virus's development, even when precise immunisation protocols are maintained (Dey *et al.,* 2019).

Vaccination effectively lowered mortality caused by IBD in the 1980s. The appearance of Delaware variations in the United States in the mid-1980s, as well as the appearance of particularly virulent strains of the virus in Europe and Asia in 1989, resulted in vaccination failures (Eterradossi & Saif, 2013). It is important for preventing infection at a young age in order to minimize the immunosuppressive effects of IBDV. This can be accomplished by immunising the parent stock. Inactivated vaccinations combination with oil adjuvants increase the immunological response, and maternal immunity can be extended for 3 to 5 weeks. When young chicks are immunised with attenuated vaccines, timing is crucial because early immunisation may result in the vaccine being neutralised by MDA, while too late vaccination may result in the chickens maintaining unprotective because of the declining level of MDA in the environment (Muller *et al.,* 2012). Monitoring the level of antibodies in a breeding flock or its progeny can determine the most efficient time to vaccinate. Vaccines can be administered intramuscularly, as a spray or mixed into drinking water. Chickens immunized with IBDV before 7 days of age and then revaccinated at 18 weeks of age with an inactivated, oil-adjuvant IBD vaccine can develop and sustain high levels of virus-neutralizing antibodies during their laying periods, which is 10 months. Furthermore, because of the early vaccination, the vaccine virus will spread throughout the chicken farm, posing a risk of immunological reactions in other vulnerable chicks (Dey *et al.,* 2019; Wagari, 2021).

5.0 CONCLUSION

In conclusion, the first outbreak of IBDV was reported in 1957 due to classical IBDV. In 1980s, variant and very virulent IBDV were initially discovered in the United States and Europe, respectively. Novel variant IBDV first emerged in China in 2017. IBDV strains (classical, variant, very virulent and novel variant) and genogroups (G1 - G7) showed some variation in clinical signs, gross and histological lesions depending on the pathogenicity of the IBDV strains and genogroups. This study reported that isolates of different of IBDV strains fall in first three genogroups (classical, variant and very virulent IBDV) whereas genogroups (G4 – G7) have mutations that are likely to contribute to altered antigenicity. Most of the genogroups are prevalent around the world, whilst the mutated and reassorted strains are found in particular region of the globe. As a result, strict biosecurity, successful vaccination programme and accurate diagnosis are important for effective prevention and control of IBDV infection.

6.0 RECOMMENDATIONS

More studies need to be done due to frequent mutation of the IBDV, therefore there are many ways to further improve this study. A new vaccine should be developed in the future for the effective prevention and control of IBDV infection.

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