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STUDIES ON LOCAL ISOLATES OF INFECTIOUS BURSAL DISEASE VIRUS

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STUDIES ON LOCAL ISOLATES OF INFECTIOUS BURSAL DISEASE VIRUS

By

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LIST OF ABBREVIATIONS

BF	- Bursa of Fabricius
CAM	- Chorioallantoic Membrane
CEF	- Chicken Embryo Fibroblast
CPE	- Cytopathic Effect
EM	- Electron Microscope
EID_{50}	- Embryo Infective Dose 50%
ELISA	- Enzyme Linked Immunosorbent Assay
HA	- Haemagglutination
HE	- Hematoxylin and Eosin
HI	- Haemagglutination-Inhibition
IBD	- Infectious Bursal Disease
IBDV	- Infectious Bursal Disease Virus
ND	- Newcastle Disease
NDV	- Newcastle Disease Virus
PBS	- Phosphate Buffer Saline
pH	- Hydrogen-ion Concentration
pi	- Post Inoculation
RNA	- Ribonucleic Acid
$TCID_{50}$	- Fifty percent Tissue Culture Infective Dose
TEM	- Transmission Electron Microscopy
UPM	- Universiti Pertanian Malaysia

Abstract of the thesis presented to the Senate of
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THU ZAR THAN @ HAFIZA HJ HASHIM

May 1996

Chairman: Dr. Mohd Hair Bin Bejo

Faculty: Veterinary Medicine and Animal Science

Infectious bursal disease (IBD) is one of the most important viral diseases in chickens. IBD virus (IBDV) from seven local field outbreaks in layer, broiler and village chickens were isolated, propagated and identified. The pathogenicity and immunogenicity of one of the selected isolates were determined in specific pathogen free (SPF) chickens.

The study showed that the IBDV can cause sudden onset of mortality ranging from 15% to 90% during the outbreaks. It occurred in both the vaccinated and non-vaccinated chickens with the age group ranging from 18 days to 14 weeks. Layers appeared to be more susceptible to the virus than the broilers, whilst the highest mortality (90%) was observed in village chickens.

Haemorrhages of the bursa of Fabricius, muscles and mucosal layers at the junction of the proventriculus and gizzard were the typical gross lesions

caused by the virus. The bursal lesions vary from moderate enlargement and oedematous to severe atrophy. Histologically, the enlarged bursa showed acute necrotizing bursitis, whilst chronic necrotizing bursitis was seen in the atrophic organ. The virus particles were detected in the cytoplasm of the lymphoid cells, macrophages and necrotic cells of the bursa of Fabricius under transmission electron microscopy (TEM). Inoculation of the IBDV isolates obtained from the bursa of Fabricius into embryonated chicken eggs caused embryonic death, haemorrhages, oedema and hepatic necrosis. The lesions were more severe in SPF eggs than the commercial eggs. Inoculation of the virus into susceptible chickens caused some variation in the mortality and lesions. All isolates of the IBDV from the outbreaks showed distinct curve of agar gel diffusion precipitation lines between the positive sample and reference serum against IBDV.

Studies on one of the isolates (UPM 93273), inoculated orally at a dose of $10^{6.8}EID_{50}$ per ml in 28-day-old SPF chickens showed that the virus was highly pathogenic and immunogenic. Sudden onset of mortality (60%) to about four times than the field outbreak occurred at days 2 and 3 post inoculation. The gross and histological lesions of the affected chickens were consistent to those of the field outbreak except that more severe lesions were observed in the SPF chickens. Acute necrotizing bursitis was observed during the early stage of the infection at days 2 to 4, while at the late stage, chronic necrotizing bursitis was recorded. The virus was detected from the bursa of Fabricius from days 1 to 10 and days 1 to 12 using embryonated chicken eggs and TEM respectively. Antibody titre against the virus was first detected at day 6 post inoculation (1271 ± 75) and reached the highest level by day 21 (4490 ± 735).

It was concluded that the study has successfully isolated and propagated a highly pathogenic strains of serotype 1 IBDV from seven field outbreaks in the country. Studies on one of the isolates demonstrated that the virus was also highly immunogenic, and thus, it can be useful as seed virus in the production of local IBD vaccine, which is safe and efficient in the prevention and control of the disease in the country.

Abstrak tesis yang dikemukakan kepada Senat
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Ijazah Master Sains

KAJIAN KE ATAS ISOLAT VIRUS TEMPATAN PENYAKIT BURSA BERJANGKIT

Oleh

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Penyakit bursa berjangkit (IBD) adalah merupakan salah satu penyakit virus terpenting pada ayam. Virus IBD (IBDV) daripada tujuh wabak penyakit tempatan yang berlaku pada ayam penelur, pedaging dan ayam kampung diasingkan, dibiakkan dan dikenalpasti. Kepatogenan dan keimunogenan satu isolat terpilih dikenalpasti dalam patogen khusus bebas (SPF) ayam.

Kajian menunjukkan IBDV boleh menyebabkan mortaliti secara tiba-tiba dalam lingkungan 15% hingga 90% semasa wabak penyakit. Ia berlaku pada ayam yang telah dan belum menerima pemvaksinan, berumur di antara 18 hari hingga 14 minggu. Ayam penelur didapati lebih mudah dijangkiti virus daripada ayam pedaging, manakala mortaliti (90%) tertinggi terdapat pada ayam kampung.

Pendarahan pada bursa Fabricius, otot, lapisan mukosa diantara proventikulus dan hempedal adalah lesi matakasar khusus jangkitan IBDV.

Lesi bursa berbeza dari sederhana membesar dan edema kepada atrofi yang teruk. Pemeriksaan histologi pada bursa yang membesar menunjukan bursitis nekrosis akut, manakala bursitis nekrosis kronik dilihat dalam atrofik bursa. Butiran IBDV didapati dalam sitoplasma sel-sel limfoid, makrofaj dan sel yang mengalami nekrosis dalam bursa Fabricius melalui mikroskop elektron pancaran (TEM). Penginokulatan IBDV isolat yang diperolehi dari bursa Fabricius ke dalam telur ayam berembrio menyebabkan kematian, pendarahan, edema dan nekrosis hati pada embrio. Lesi embrio di telur SPF lebih teruk dibandingkan dengan di telur komersial. Inokulasi IBDV ke dalam ayam menunjukkan sedikit perbezaan mortaliti dan lesi di antara isolat. Semua isolat IBDV tempatan menunjukkan keluk jelas ujian sebaran pemendakan gel agar (AGPT) di antara sampel positif dan sampel serum rujukan IBDV.

Kajian pada satu isolat (UPM 93273) yang diinokulat melalui mulut pada dos $10^{6.8}$ EID_{50} setiap ml pada ayam SPF berumur 28 hari menunjukkan IBDV isolat tersebut sangat patogenik dan imunogenik. Mortaliti (60%) secara tiba-tiba sehingga mencapai empat kali ganda dari kejadian wabak di ladang berlaku pada hari kedua dan ketiga post inokulasi. Lesi matakasar dan histologi pada ayam yang terlibat adalah seperti lesi sewaktu wabak penyakit, kecuali lesi yang lebih teruk terdapat dalam ayam SPF. Bursitis nekrosis akut dilihat di peringkat awal jangkitan virus pada hari kedua hingga keempat, manakala di peringkat akhir, bursitis nekrosis kronik berlaku. Virus IBD dapat dikesan di bursa Fabricius dari hari pertama sehingga hari keduabelas dengan menggunakan TEM dan hari pertama sehingga hari

kesepuluh melalui telur ayam berembrio. Titre antibodi IBDV mula dikesan pada hari keenam post inokulasi (1271$\pm$75) dan mencapai tahap tertinggi pada hari ke 21 (4490$\pm$735).

Kesimpulannya, kajian ini telah berjaya mengasing dan membiakkan serotaip 1 IBDV strain yang sangat patogenik daripada tujuh wabak penyakit yang berlaku di negara ini. Kajian pada salah satu isolat tersebut menunjukkan ianya juga sangat imunogenik. Isolat ini mungkin boleh digunakan sebagai benih virus dalam pengeluarkan vaksin IBD tempatan yang selamat dan dapat memberi perlindungan sepenuhnya dalam pencegahan dan kawalan wabak IBD di negara ini.

CHAPTER 1

INTRODUCTION

Infectious bursal disease (IBD) or Gumboro disease, an extremely important viral disease in poultry industry worldwide, was first reported in the district of Delaware in the U.S.A in 1957 (Cosgrove, 1962). However, the clinical outbreaks of the disease was only reported in Malaysia in 1991 (Hair-Bejo, 1992; Loganathan *et al.*, 1992). Since then, acute IBD outbreaks were diagnosed in both layer and broiler chickens in the country. The disease causes intense economic losses due to sudden onset of high mortality and high condemnation of the carcasses or resulting from high mortality associated with secondary infections and failure to the vaccination programmes against other highly virulent diseases (Hair-Bejo, 1994).

Infectious bursal disease virus (IBDV) is a bisegmented double-stranded RNA (dsRNA) virus with icosahedral symmetry and a diametre of about 50 to 55 nm (Muller *et al.*, 1979b; Dobos *et al.*, 1979; Becht, 1980; Jackwood *et al.*, 1984). Two distinct serotypes of IBDV designated as serotype 1 within that occurred antigenic variants, and serotype 2 were recognized (McFerran *et al.*, 1980; Jackwood *et al.*, 1982; McNulty and Saif, 1988; Wood *et al.*, 1988). Only serotype 1 IBDVs have been known to cause naturally occurring disease in chickens. It occurs as a clinical or subclinical IBDV infection (Rosenberger and Gelb, 1978). The clinical IBD usually occurs in chickens

infected with either standard or highly virulent strains of serotype 1 IBDV in birds more than 2-3 weeks old (Cosgrove, 1962; Parkhurst, 1962; Winterfield and Hitchner, 1964; Luthgen, 1969), whilst infection in the younger age could lead to subclinical IBD. The subclinical IBD is also observed in chicks infected with variant strains of the virus (Snyder, 1990).

IBDV replicates primarily in the lymphoid cells of the bursa of Fabricius (Ismail *et al.*, 1987; Okoye and Uzoukwu, 1990). It induces severe necrosis of the lymphocytes and atrophy of the organ (Hirai *et al.*, 1974). The availability of the large number of bursal cells (B cells) has been shown to be an essential factor in development of IBD infection (Ismail *et al.*, 1987).

Isolation of IBDV from the field outbreaks may be difficult, although the virus was successfully isolated in embryonated chicken eggs (Winterfield *et al.*, 1962) with the chorioallantoic membrane (CAM) route performed the best for the virus isolation. Embryo
(Hitchner, 1978). The virus also has been isolated using chicken embryo fibroblast cell cultures (Lukert and Davis, 1974; McNulty *et al.*, 1979; Lee and Lukert, 1986), bursa B-cell derived lymphoblastoid cell line (Hirai and Calnek, 1979) and BGM-70 cell line (Jackwood *et al.*, 1987).

IBDV is very stable to chemical and physical agents and it can remain for long in a contaminated environment (Benton, 1967b). Once the infection set

up on a farm, it recurs in several subsequent flocks despite the most thorough cleansing and disinfection (Winterfield and Hitchner, 1964; Edgar and Cho, 1973; Hair-Bejo *et al.*, 1995a). There is no therapeutic measures for IBDV infection and the disease can only be prevented by proper immunization programmes in both the parent stock and their progeny.

To date, about 46 types of IBD vaccines were imported for use in West Malaysia (Chin, 1993). Several vaccination programmes against the disease has been carried out, but their success are unpredictable (Hair-Bejo, 1993). Outbreaks of IBDV continued to occur in both the vaccinated and non-vaccinated chickens in the country (Hair-Bejo, 1994). The safety and efficacy of the vaccines under local conditions is little understood. Studies on some of the vaccines showed that the IBD vaccines can induce lesion similar to those observed in the field infection (Hair-Bejo *et al.*, 1994). The failure of IBD vaccines to induce antibody against IBDV was also reported (Hair-Bejo *et al.*, 1995c). Furthermore. the emergence of different strains of IBDV in the country may complicate the immunization programme of the disease.

Thus, it is an urgent need to study on local isolates of IBDV for the prevention and control of the disease in the country. The highly pathogenic IBDV isolates can be used for challenge virus in the study of the safety and efficacy of IBD vaccines, whilst the highly immunogenic viruses is needed for virus seed in the production of local IBD vaccine which is safe and provides

full protection against the IBDV field challenge. Therefore, the objectives of this study are:

1. to isolate propagate and characterize the recent field isolates of IBDV, and
2. to determine the pathogenicity and immunogenicity of a selected IBDV local field isolate.

CHAPTER 2

LITERATURE REVIEW

Background

Infectious bursal disease (IBD) was first reported in a broiler flock in the small village, Delaware, USA in 1957 (Cosgrove, 1962). The common synonyms of the disease are avian nephrosis (Cosgrove, 1962), Gumboro disease (Faragher, 1972), infectious bursitis (Ibragimov, 1976) and avian infectious bursitis (Rodon, 1982). Since the first outbreak of IBD, the disease was then reported from most major poultry producing areas around the world such as in Europe (Faragher, 1972), Australia (Firth, 1974), India (Mohanty *et al.*, 1971) and Japan (Shimizu *et al.*, 1971).

Serious outbreaks of clinical IBD with high mortality, up to 90% due to highly virulent strains of serotype 1 IBD virus (IBDV) occurred in late 1980's throughout Europe (Chettle *et al.*, 1989; Van den Berg *et al.*, 1991). The disease had spread worldwide and was described in Asia in 1990's (Nunoya *et al.*, 1992) including Malaysia in 1991 (Hair-Bejo, 1992, Loganathan *et al.*, 1992). It occurred in both the vaccinated and non-vaccinated chickens and the mortality increased with the presence of concurrent infections (Hair-Bejo, 1994).

Infectious Bursal Disease Virus

Classification

Infectious bursal disease virus was first classified as a picornavirus (Cho and Edgar, 1969). To date, IBDV is referred as a Diplorna virus, a member of the newly established genus birna virus under family Birnaviridae with a genome consisting of a bisegmented double-stranded RNA (dsRNA) (Dobo *et al.*, 1979; Brown, 1984). Two serotypes of IBDV, serotypes 1 and 2, have been recognized. Serotype 1 IBDV is pathogenic to chicken whereas serotype 2 virus is isolated from turkey (McFerran *et al.*, 1980; Jackwood *et al.*, 1982; Cummings *et al.*, 1986) and non pathogenic in chickens (Ismail *et al.*, 1987). The strains of serotype 1 IBDV has been tentatively classified into three groups based on the pathogenicity and antigenicity; very virulent strains of serotype 1 IBDV (vvIBDV) (Chettle *et al.*, 1989; Van den Berg *et al.*, 1991; Nunoya *et al.*, 1992; Tsukamoto *et al.*, 1992), standard strains of serotype 1 IBDV (stIBDV) (Hirai *et al.*, 1973; Sharma and Lee, 1984; Chettle *et al.*, 1989; Craft *et al.*, 1990), and variant strains of serotype 1 IBDV (vaIBDV) (Saif, 1984; Rosenberger and Cloud, 1985; Sharma *et al.*, 1989; Craft *et al.*, 1990; Snyder, 1990).

Morphology

Negatively stained IBDV particles examined under transmission electron microscopy (TEM) demonstrated that the virus is a non-enveloped, single capsid

shell of icosahedral symmetry varying from 55 to 65 nm (Hirai and Shimakura, 1974; Hirai *et al.*, 1979; Dobo *et al.*, 1979; Brown, 1984). The virus varies somewhat in appearance depending upon the purification procedure employed. Local pH and ionic concentrations affect the state of hydration of the virion and this has the morphological consequences described (Harkness *et al.*, 1975).

IBDV particles arrayed in a crystalline pattern measured about 50-55 nm was detected in the cytoplasm of lymphoid cells, macrophages, heterophils and reticular epithelial cells of the bursa of Fabricius examined directly under TEM (Kaufer and Weiss, 1976; Hair-Bejo, 1993). Immature forms of IBDV was first found in the cytoplasmic inclusion bodies at 24 hours post inoculation, whilst relatively large amounts of mature viruses are present in macrophage inclusions by day 3 (Kaufer and Weiss, 1976).

Buoyant density of IBDV particles in cesium chloride (CsCl) gradients has been reported to be 1.33 g/cm^3 (Nick *et al.*, 1976; Becht, 1980; Muller and Becht, 1982; Fahey *et al.*, 1985). A higher buoyant density of 1.34 g/ml (Hirai *et al.*, 1979) and lower densities of 1.31 g/ml (Pattison *et al.*, 1975; Todd and McNulty, 1979) and 1.32 g/ml (Jackwood *et al.*, 1982, 1984) have also been reported. Immature (incomplete) virus particles have buoyant densities lower than 1.33 g/ml in CsCl gradients.

Resistance to Chemical and Physical Agents

IBDV is relatively refractory to heat, ultraviolet irradiation and photodynamic inactivation (Petek *et al.*, 1973). It is inactivated at pH 12.0, but