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THE PATHOGENICITY OF VELOGENIC VISCEROTROPIC NEWCASTLE DISEASE VIRUS IN THE BURSA OF FABRICIUS

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by

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LIST OF ABBREVIATIONS

AC	after challenge
AGPT	agar gel precipitation test
cm	centimetre
°C	degrees centigrade
gm	gramme
GMT	geometric mean titre
HA	haemagglutination
H & E	hematoxylin and eosin
HI	haemagglutination inhibition
IBD	infectious bursal disease
IBDV	infectious bursal disease virus
i/c	in-contact
ILT	infectious laryngotracheitis
ILTV	infectious laryngotracheitis virus
GLS	gross lesion score
MD	Marek's disease
MDV	Marek's disease virus
ml	millilitre
mm^3	millimetre cube
MMLS	mean microscopic lesion score
ND	Newcastle disease
NDV	Newcastle disease virus
NVCO	non-vaccinated control
NVC	non-vaccinated challenged
%	percent
SEM	scanning electron microscope
TEM	transmission electron microscope
ul	microlitre
VCO	vaccinated control
VC	vaccinated challenged
VVNDV	velogenic viscerotropic Newcastle disease virus

Abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Master of Science.

PATHOGENICITY OF VELOGENIC VISCEROTROPIC NEWCASTLE DISEASE VIRUS IN THE BURSA OF FABRICIUS

by

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Faculty : Veterinary Medicine and Animal Science

Newcastle disease (ND) is one of the important poultry diseases in Malaysia. Although the disease is controlled through vaccination programmes, outbreaks have been reported in vaccinated flocks from time to time. The success of any vaccination programme depends on several factors and one of them is the integrity of the immune system. The bursa of Fabricius is an important organ of the immune system which is responsible for humoral immunity against ND. Realising its importance, a study was undertaken to investigate the effect of velogenic viscerotropic Newcastle disease virus (VVNDV) on the bursa of Fabricius. An initial detailed study on the normal structure of the bursa of Fabricius was conducted as a basis for differentiating the effect of VVNDV on the bursa of Fabricius.

The effect of VVNDV on the bursa of Fabricius of vaccinated chickens was determined by infecting these chickens via contact with non-vaccinated chickens challenged intranasally with 0.1 ml of inoculate containing 10^6 EID_{50} of the virus. The damage to the bursa of Fabricius was examined by histological and electron microscopic studies, while virus was detected by isolation, immunoperoxidase staining and transmission electron microscopy.

The results showed that VVNDV damaged and replicated in the bursa of Fabricius of vaccinated birds. Grossly, the bursa was swollen, oedematous, haemorrhagic and necrotic. Histopathology showed that there were haemorrhages, oedema, cystic cavities in the follicles containing mucous, necrosis of the follicles and presence of a reduced number of lymphocytes and an increased number of heterophils, macrophages and plasma cells. Scanning electron microscopy showed increased mucous secretion and there was exfoliation of the microvilli. However the follicle-associated-epithelium was intact in the vaccinated challenged birds. In the non-vaccinated challenged birds there was total exfoliation of the epithelium. Viral isolation and immunoperoxidase staining showed evidence of viral replication in the bursa of Fabricius. Ultrastructurally, viral replication was shown by the presence of virus budding and nucleocapsids in the cytoplasm of lymphoid cells and macrophages.

This is the first time such a study was undertaken in Malaysia. The information obtained from this study is very important as VVNDV is endemic in this country. The damage to the bursa of Fabricius by VVNDV may have serious consequense to the success of vaccination against other diseases. In Malaysia besides ND, such as infectious bronchitis, fowl pox, Marek's disease and infectious

bursal disease are also controlled by vaccination. Damage to the bursa of Fabricius of ND vaccinated chickens by VVNDV may reduce the immune response to other vaccinations.

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KEPATOGENAN VIRUS PENYAKIT SAMPAR AYAM VELOGENIK VISEROTROPIK PADA BURSA FABRICIUS

oleh

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Sampar ayam adalah satu penyakit ayam yang penting di Malaysia. Walaupun penyakit ini dikawal melalui program pemvaksinan, wabak telah di laporkan berlaku dalam kelompok ayam yang telah diberi vaksin dari semasa ke semasa. Kejayaan sesuatu program pemvaksinan bergantung kepada beberapa faktor dan satu daripadanya ialah kesempurnaan sistem imun. Dalam hubungan ini bursa Fabricius adalah penting dan bertanggungjawab ke atas imuniti humor melawan ND. Menyedari kepentingan bursa Fabricius dalam sistem imun, satu kajian telah dijalankan untuk meneliti kesan virus penyakit sampar ayam velogenik viserotropik (VVNDV) ke atas bursa Fabricius ayam yang telah diberi vaksin. Di peringkat awal, satu kajian terperinci struktur normal bursa Fabricius telah dijalankan untuk memberi kefahaman asas kesan VVNDV pada bursa Fabricius ayam yang telah diberi vaksin.

Kesan VVNDV pada bursa Fabricius ayam yang telah diberi vaksin telah dijalankan dengan menginfeksi ayam-ayam tersebut secara sentuhan dengan ayam-ayam yang tidak diberi vaksin dan dicabar melalui hidung dengan 0.1 ml inokulat mengandungi 10^6 EID_{50} VVNDV. Kerosakan pada bursa Fabricius telah diperiksa melalui kajian histopatologi dan mikroskopi elektron manakala pembiakan virus telah dikesan melalui pemencilan virus, immunoperoksidase dan mikroskopi elektron pancaran.

Hasil kajian ini menunjukkan VVNDV merosakkan dan membiak dalam bursa Fabricius ayam yang telah diberi vaksin. Dari pandangan mata kasar, bursa membengkak, beredema, berhemoraj dan nekrotik. Histopatologi menunjukkan adanya hemoraj, edema, kaviti sista dalam folikel mengandungi mukus, nekrosis dalam folikel dan adanya pengurangan limfosit dan bertambahnya heterofil, makrofaj dan sel plasma. Mikroskop elektron pembias menunjukkan bertambahnya rembesan mukus, mikrovilus tertanggal tetapi epitelium masih tidak rosak pada bursa ayam yang diberi vaksin. Pada ayam yang tidak diberi vaksin dan dicabar, keseluruhan epitelium tertanggal. Pembiakan virus adalah ketara dikesan pada bursa ayam yang diberi vaksin sepanjang tempoh cabaran, dipastikan secara pemencilan virus dan perwarnaan immunoperoksidase. Dari segi ultrastruktur, pembiakan virus adalah ketara dengan adanya virus bertunas dan nukleokapsid dalam sitoplasma sel limfoid dan makrofaj.

Kajian ini adalah yang pertama dijalankan di Malaysia. Penerangan yang didapati dari kajian ini sangat penting kerana VVNDV adalah endemik di negara ini. Kerosakan pada bursa Fabricius oleh VVNDV akan mempunyai kesan yang serius ke atas kejayaan pemvaksinan melawan lain-lain penyakit. Di Malaysia

selain daripada ND, lain-lain penyakit seperti bronkitis berjangkit, puru ayam, penyakit Marek dan penyakit bursa berjangkit adalah juga dikawal melalui pemvaksinan. Kerosakan oleh VVNDV pada bursa Fabricius ayam yang telah diberi vaksin ND, akan mengurangkan tindakbalas pemvaksinan.

CHAPTER 1

INTRODUCTION

Newcastle disease is (ND) one of the important viral diseases of poultry in the world. It occurs in most countries and is the cause of heavy economic losses to poultry farmers particularly in the developing countries.

Newcastle disease virus (NDV) is a member of the family Paramyxoviridae. The virion is pleomorphic, spherical or filamentous with a diameter of 150 to 300 nm or more. It consists of a lipid-containing bilayer envelope with glycoprotein peplomers surrounding a helically symmetrical "herringbone" nucleocapsid with a diameter ranging from 12 to 18 nm in different genera (Fenner *et al.*, 1987).

NDV infection in chicken varies from a mild subclinical infection (Beard, 1971) to a severe peracute fatal disease with or without lesions (Hanson, 1978). The pathogenicity of NDV depends on the host as well as the pathotype of NDV; it could either be lentogenic, mesogenic or velogenic (Lancaster and Alexander, 1975). Of the three pathotypes of NDV the velogenic viscerotropic Newcastle disease virus (VVNDV) has been considered to possess the greatest virulence (Utterback and Schwartz, 1973). This pathotype is commonly found in South East Asia. VVNDV is responsible for high morbidity and mortality of chickens in Malaysia.

The immune system plays an important role in the defence mechanism of the chicken against NDV. Defects in the integrity of the bursa of Fabricius and the thymus may predispose the chicken to secondary infection. In NDV infection humoral immunity plays a major role and the bursa of Fabricius is the main lymphoid organ. Pathogens like NDV which interfere with the bursa of function would eventually affect the immune system of chicken.

The immune system of chicken comprises of two morphologically and functionally distinct components, the bursa of Fabricius and the thymus (Cooper *et al.*, 1965, 1966; Clawson *et al.*, 1967; Durkin *et al.*, 1972). The bursa of Fabricius is important in the development of immunocompetence in the chicken (Cooper *et al.*, 1965, 1966; Clawson *et al.*, 1967; Warner and Szenberg, 1964; Naukkarinen and Sorvari, 1984). It acts as a central organ for the differentiation of B lymphocytes, and the presence of a functional bursa is essential for the development of B-cell dependent antibodies in the young chick.

Many studies on the importance of the bursa of Fabricius in immunity against Newcastle disease have been carried out (Hirato and Bito, 1975; Matsuda and Bito, 1973; Sulochana and Jayaprakasan, 1983; Mishra and Jaiswal, 1984). However, very few studies have been done, on the effect of VVNDV on the bursa of Fabricius.

To understand the effect of VVNDV on the bursa of Fabricius, an understanding of the normal structure of the bursa of Fabricius is essential. As the bursa is an important organ in the humoral immune system of chicken, impairment or destruction of the organ will predispose the chicken to other diseases.

The hypothesis of this study is that VVNDV can infect the bursa of vaccinated chickens.

Thus the objectives of this study are:

1. to study the normal morphology of the bursa of Fabricius.
2. to determine the effect of VVNDV in the bursa of vaccinated and non-vaccinated chickens.

CHAPTER 2

LITERATURE REVIEW

Newcastle Disease Virus

Newcastle disease virus (NDV) is the cause of a world-wide serious disease in poultry. There are three pathotypes of NDV: the lentogenic, mesogenic and velogenic strains (Hanson and Brandly, 1955; Allan *et al*., 1978). The lentogenic strain is the least virulent. This type causes a significant drop in egg production but a very low rate of mortality. The mesogenic strain is of moderate virulence, also causing drastic reduction of egg production but the mortality rate is about 50%. The velogenic strain being the most virulent, causes severe disease with high mortality rates in chickens (Spradbrow, 1987).

Newcastle Disease Virus Structure

The virions of NDV have a core of helical nucleocapsid consisting of a strand of ribonucleic acid (RNA) surrounded by protein, which is enclosed within a lipoprotein membrane with lipid of host origin (Horne *et al.*, 1960; Cruikshank, 1964; Kingsbury and Darlington, 1968). Negative contrast electron microscopy of NDV virions show pleomorphic particles usually ranging from 100-300 nm in diameter but sometimes as large as 600 nm diameter (Waterson and Cruikshank, 1963; Andrews and Pereira, 1972; Lai, 1985).

Pathogenicity of Newcastle Disease Virus

The pathogenicity of NDV depends on three basic factors: the host, virus and environment. The host factor is determined by the breed of chicken (Cole and Hutt, 1961; Francis *et al.*, 1964), the age of the chicken (Cole and Hutt, 1961; Lancaster, 1966; Chu and Rizk, 1975) and the immune status of chicken (Chu and Rizk,1975). Pathogenicity is also determined by the strain of the virus (Sinha *et al.*, 1952), the dose of the infecting virus (Karzon and Bang, 1951) and the portal entry of the virus (Karzon and Bang, 1951; Kohn, 1955; Beard and Easterday, 1967). Environmental factors such as heat, cold, trauma and overcrowding are as important as the other two factors in determining the pathogenicity of NDV (Cheville, 1978).

Infection with Newcastle Disease Virus

NDV enters the host through the mouth and the nostrils, then it replicates in the mucosal epithelium of the upper respiratory and intestinal tracts. Shortly after infection, during the primary viraemia, the virus spreads via the blood to the spleen and bone marrow, later producing a secondary viraemia (Fenner *et al.*, 1987). This leads to infection of other target organs such as lung, intestine, caecal tonsils, kidney, liver, bursa and central nervous system (Cheville *et al.*, 1972 b; Parede and Young, 1990).

The severity of NDV infection varies depending on the pathotype. Infection with lentogenic strains may result in tissue damage and high titres of virus at sites of initial

infection, that is at the epithelium of the intestine or upper respiratory tract, but the virus does not replicate in or cause lesions of visceral target organs (Beard and Easterday, 1967). In contrast, highly virulent velogenic strains rapidly cause fatal systemic disease after entry through epithelial surfaces (Jungherr *et al.*, 1946). Cheville *et al.*, (1972 b) supported this concept by reporting that the replication of lentogenic strain of NDV is limited to the respiratory epithelium whereas virulent NDV replicates in the reticuloendothelial system, brain, heart, liver, kidneys and most other organs.

Bang (1953) suggested that avirulent strains of NDV mature at the cell surface whereas virulent strains are formed within the cytoplasm. This was partially supported by Cheville *et al.* (1972 a) who reported that the less virulent B1 and Ulster strains do mature at the surface of the allantoic epithelium but they could not confirm that virulent NDV strains mature chiefly within the cytoplasm.

Infection with VVNDV will cause almost 100% mortality in non-NDV vaccinates whereas almost no morbidity or mortality but an increase in antibody titres occurs in NDV vaccinates (Aini, 1989 ; Xie and Stone, 1990).

Newcastle Disease Virus Replication

NDV replication is initiated by the adsorption of the virus to the cell surface. This process is mediated by the attachment of haemagglutinin of the virus to glycoprotein receptors on the cell surface (Kohn, 1965; Reeve *et al.*, 1970). Then the virus envelope and the cell membrane fuse; subsequently, the nucleocapsid is extruded into the cell.