UNIVERSITI PUTRA MALAYSIA

A STUDY ON A VELOGENIC VISCEROTROPIC NEWSCASTLE DISEASE VIRUS IN VITRO AND IN VIVO

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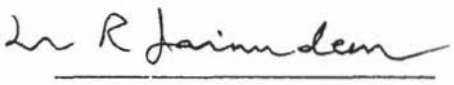

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IN VITRO AND *IN VIVO*

by

Lai Chooi May

A thesis submitted in partial fulfilment of the
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TO CHEE-YIN AND ELAINE

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An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

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by

Lai Chooi May

April, 1985

Supervisor : Professor Abdul Latif bin Ibrahim

Faculty : Veterinary Medicine and Animal Science

The velogenic viscerotropic Newcastle disease virus or the Asiatic strain has been considered the most virulent strain of Newcastle disease virus. It is commonly found in Southeast Asia and it has been known to cause 100% mortality in susceptible flocks. In spite of this, very little research has been conducted on it as many countries prohibit the handling of this strain of virus. In view of this, a project has been undertaken at Universiti Pertanian Malaysia to study the biological properties, cytopathogenicity and morphogenesis of a locally isolated velogenic viscerotropic Newcastle disease virus and to determine its effects on the trachea of nonvaccinated and vaccinated chickens.

The in vitro study has shown that this virus has a mean death time of 66 hours, and an intracerebral pathogenicity index of 1.90. Polykaryocytosis is the principal form of cytopathic effect it produces in chick embryo fibroblasts and cells infected by it haemadsorp red blood cells. This virus plaques in cell culture. Negatively stained virus particles have diameters

ranging from 100 to 600 nanometers. Electron microscopy demonstrated that the virus replicates in the cytoplasm of infected cells and aggregates of nucleocapsids are found in the cytoplasm. The virus matures at the cell membrane and is released by budding.

The effects of the virus on the tracheal epithelium was evaluated by virus isolation and scanning and transmission electron microscopy. This virus causes severe ultrastructural alterations in the nonvaccinated chickens and it could be isolated from the tracheas of all the infected chickens from day 4 postinfection. None of these chickens survived past day 10 postinfection. In the vaccinated chickens, differences were observed. Besides a delay in the onset and a decrease in severity of the damages, the vaccinated chickens were apparently normal throughout the experiment with no death recorded and virus could only be isolated from some of the chickens from day 6 to 9 postinfection. Complete regeneration of the damaged epithelium was accomplished by day 13 postinfection.

This study presents for the first time detailed information on the *in vitro* properties of a locally isolated velogenic viscerotropic Newcastle disease virus. Such information is useful in differentiating the virus from the vaccine strains present, thereby assisting in the diagnosis of the disease. It also provides a basis for understanding the behaviour of the virus in the host. Information on its replication at the site of entry as well as on the response of the vaccinated and nonvaccinated chickens to the virus suggest not only the importance of vaccination but also the call for a good vaccination programme in the control of the disease. The study has also thrown light on the possible

epidemiology of the virus in relation to the poultry industry in Malaysia.

Abstrak daripada tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia untuk memenuhi sebahagian dari keperluan untuk Ijazah Doktor Falsafah.

KAJIAN *IN VITRO* DAN *IN VIVO* VIRUS SAMPAR AYAM VELOGENIK VISEROTROPIK

Oleh

Lai Chooi May

April, 1985

Penyelia: Profesor Abdul Latif Ibrahim

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Virus sampar ayam velogenik viscerotropik atau strain Asia dianggap sebagai jenis virus sampar ayam yang paling virulen. Virus ini biasanya didapati di Asia Tenggara dan dapat mengakibat kematian 100% dalam kelompok ayam yang suseptibel. Walaubagaimanapun, penyelidikan mengenai virus tersebut tidak banyak dijalankan. Ini disebabkan banyak negara melarang menjalan kajian mengenai virus ini. Memandangkan hal demikian, satu projek telah dijalankan di Universiti Pertanian Malaysia untuk mengkaji ciri biologi, sitopatogenisiti dan morfogenesis virus sampar ayam velogenik viscerotropik yang telah diasingkan di negara ini. Projek ini juga mengkaji kesan virus tersebut pada trakea ayam yang sudah disuntik dan tidak.

Kajian *in vitro* menunjukkan bahawa virus ini mempunyai purata masa kematian 66 jam dan indeks patogenisiti intraserebrum 1.9. Polikaryositosis merupakan bentuk sitopatik utama yang

didapati pada fibroblast embrio ayam dan sel yang dijangkiti hemadsorp sel darah merah. Virus tersebut membentuk plak pada kultura sel. Partikel virus yang diwarna negatif mempunyai diameter diantara 100 hingga 600 nanometer. Mikroskopi lektron menunjukkan virus tersebut membiak dalam sitoplasma sel yang dijangkiti dan agregat nukleokapsid didapati dalam sitoplasma. Virus tersebut matang dekat diselaput sel dan bebas secara penunasan.

Kesan virus tersebut kepada epitelium trakea telah dinilai dengan pengasingan virus dan mikroskopi lektron transmissi dan skanning. Virus tersebut menyebabkan perubahan ultrastruktur pada ayam yang tidak disuntik dan virus dapat diasing dari trakea semua ayam yang dijangkiti mulai hari ke 4 selepas dijangkiti. Tiada seekor ayam pun yang hidup lebih daripada hari ke 10 selepas dijangkiti. Perbezaan dapat dilihat pada ayam yang di suntik. Selain daripada permulaan kerosakan yang lambat dan pengurangan tahap kerosakan, ayam yang disuntik kelihatan sehat tanpa apa-apa tanda sakit. Tiada kematian berlaku dan virus hanya dapat diasing daripada beberapa ekor ayam pada hari ke 6 hingga 9 selepas dijangkiti. Regenerasi yang lengkap pada sel yang mengalamai kerosakan dicapai pada hari ke 13 selepas jangkitan.

Kajian ini ialah yang pertama kalinya memberi maklumat yang lengkap mengenai ciri *in vitro* virus sampar ayam velogenik viscerotropik yang diasingkan di negara ini. Maklumat tersebut berguna untuk membezakan virus ini daripada virus strain vaksin. Ini dapat membantu dalam diagnosa penyakit ini. Ciri *in vitro* virus ini juga memberi asas untuk mengetahui kelakuan virus ini pada perumahnya. Maklumat mengenai pembiakan virus di tempat masuk dan juga respon ayam yang sudah disuntik dan yang tidak terhadap

virus itu bukan sahaja menunjukkan ayam perlu disuntik, tetapi satu program suntikan yang baik untuk mengawal penyakit tersebut perlu dirancang. Kajian ini menunjukkan kemungkinan epidemiologi virus ini ada hubungan dengan perusahan ayam di negara ini.

CHAPTER 1

INTRODUCTION

Newcastle disease (ND) is a very important disease of chickens in many parts of the world. The disease is highly infectious and contagious and it is characterized by high mortality and morbidity, loss in body weight and drop in egg production. ND was first reported near Batavia, on the island of Java in Indonesia, by Kraneveld in 1926 (Brandly, 1964). The mortality rate was very high and within six months, the infection spread over the entire island. In the same year, this disease was recognised in two other widely separated places; on a poultry farm near Newcastle-on-Tyne in England by Doyle (1927) from which the common name of the disease is derived, and in Korea by Konno et al (1929) (cited in Brandly ,1964) who described it as an acute and usually fatal respiratory and nervous disease.

Within three years of the first outbreak, ND spread throughout the whole of Southeast Asia (Lancaster, 1966) which has now become the home of ND virus (NDV), Australia (Johnstone, 1931) and India (Brandly ,1964). In the United States, ND was not recognised until 1944 (Beach, 1944) in California although there is good evidence to believe that the NDV had been present for some years before that date. This delay in identification could be attributed to the difference between the American and the Asian and English outbreaks : the American ND was much milder, often with low mortality, longer clinical course and less pronounced haemorrhagic, inflammatory and necrotic lesions in the