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EPIDEMIOLOGY OF AUJESZKY'S DISEASE IN PIGS IN MALAYSIA

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BY

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DEDICATION

To my wife and family for their constant encouragement and support

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LIST OF ABBREVATIONS

AD	-	Aujeszky's disease
BMDP	-	Biomedical Data Programme
CPE	-	cytopathic effect
CPEA	-	Computer Programmes for Epidemiologic Analysis
DNA	-	deoxyribonuclease acid
DVS	-	Department of Veterinary Services
ELISA	-	enzyme-linked immunosorbent assay
gE	-	glycoprotein E
g	-	gravity, gramme
i.e.	-	that is
L	-	litre
M	-	molar
nm	-	nanometres
OD	-	optical density
p	-	confidence level
PBS	-	phosphate buffered saline
RNA	-	ribonucleic acid
SNT	-	serum neutralization test
S/P	-	sample to positive
ST	-	swine testicles
$TCID_{50}$	-	50% tissue culture infective dose
µg	-	microgramme
µl	-	microlitre
>	-	more or equal
<	-	less or equal

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EPIDEMIOLOGY OF AUJESZKY'S DISEASE IN PIGS IN MALAYSIA

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Aujeszky's disease (AD) causes heavy mortalities among young pigs and great economic loss in pig farms. Despite vaccination, frequent sporadic outbreaks have been reported in different parts of Malaysia. The extent of economic loss caused by the disease is not clearly known since no detailed study has been conducted to measure the prevalence and impact of the disease in pig production. This study reports the results of a nationwide sero-epidemiological survey of the disease and attempts to determine its status among the pig population in Malaysia. It also examines some control measures that can be taken to reduce the prevalence of the disease.

A commercial glycoprotein E (gE) negative enzyme-linked immunosorbent assay (ELISA) kit was used in the serological survey. The kit could detect antibodies against gE of AD virus which is present in the field strain of the virus

but not in the vaccine strain. In addition, it could test large number of serum samples in a short period of time. The ELISA was evaluated with respect to the serum neutralization test (SNT) which was used as the standard reference test. The kappa value between the ELISA S/N values and SNT titres were found to be 0.95, indicating that there was a good agreement between the ELISA and SNT. It was also found that the ELISA had a high index of sensitivity (96.25%) and specificity (98.75%) comparable to SNT. The investigations therefore, confirmed the suitability of the ELISA as a practical alternative to the SNT as a mass screening test for the serodiagnosis of AD.

To investigate the sero-epidemiology of AD in pigs in the country, a total of 2985 blood samples were collected from 100 pig farms in six major pig producing states of Malaysia and screened for gE antibodies against AD virus using the gE ELISA kit. Collectively, the pig population of these farms accounted for about 20.3 per cent of the pig population in West Malaysia. A widespread occurrence of the infection in the pig population in Malaysia was detected due to the high percentage (55.4%) of serological reactors in 84% of the farms surveyed. The study confirmed that AD is highly prevalent among pig herds in Malaysia and that vaccination on an individual herd basis did not minimize the spread of the virus among breeding pigs in enzootically infected, high pig density regions. To reduce the prevalence of AD, vaccination in the finishing section of farrow-to-finish herds may be warranted using more effective vaccines. In addition, an effective vaccination programme and a better biosecurity management system in the farms may be necessary to reduce

the prevalence of the disease to a point where an eradication programme against AD could be initiated in Malaysia.

The immune response in pigs against a disease has been known to be maximum when vaccination is given at the time of lowest maternal antibody level. To determine the optimum time of vaccination against AD in piglets in an endemic farm, blood samples were collected randomly from pigs of ages 4 to 16 weeks. The blood samples were tested for antibodies against AD by the SN test. It was found that maternal antibodies against AD persisted in pigs up to 14 weeks of age. Therefore, earlier vaccination may not be effective as the maternal antibodies may interfere with the vaccine virus. In another investigation, it was found that piglets born from parity three dams had higher levels of maternal antibodies against AD than piglets born of parity one sows. Vaccination for piglets born of dams of higher parity may therefore, be preferably at a later date than piglets born from dams of lower parity.

A study using a live and an inactivated AD vaccine was conducted in four-week-old pigs in a commercial farm to determine the effect of maternally derived antibodies on vaccination against endemic AD. It was found that maternally derived antibodies did not have a profound effect on vaccination against AD in four-week-old piglets. Furthermore, there were less economic losses in terms of mortality and body weight in vaccinated pigs as compared to non-vaccinated animals. Economic losses were lowest in pigs vaccinated with the live vaccine.

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Oleh

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Pengerusi : **Profesor Madya Dr. Henry Too Hing Lee**

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Penyakit Aujeszky (AD) menyebabkan kadar kematian yang tinggi di kalangan anak babi dan mengakibatkan kerugian yang amat besar dalam peternakaan babi. Walaupun pemvaksinan dijalankan, penyakit ini masih terjadi di beberapa kawasan di Malaysia. Kerugian ekonomi yang disebabkan oleh penyakit ini kurang jelas kerana sehingga kini tidak ada suatu kajian terperinci mengenai prevalens dan impak penyakit tersebut kepada industri babi di Malaysia. Kajian ini melaporkan hasil penyelidikan sero-epidemiologi AD di Malaysia dan bertujuan untuk menentukan status penyakit tersebut. Ia juga mengkaji beberapa langkah pengawalan yang dapat diambil untuk mengurangkan prevalens penyakit tersebut.

Dalam kajian ini, ujian assai enzim-gabung immunosorbent (ELISA) yang berkomersial telah digunakan. Ujian ini dapat mengesan antibodi terhadap glycoproten E (gE) yang didapati di dalam virus strain lapangan AD tetapi yang tidak didapati dalam virus strain vaksin. Dengan cara ini, seroprevalens antibodi

strain lapangan AD dapat dikesan. Disamping itu, ujian ELISA ini juga dapat menguji sampel darah yang banyak dalam jangkamasa yang singkat. Ujian ELISA ini telah dinilai dengan menggunakan ujian peneutralian serum (SNT) sebagai ujian rujukan dan adalah didapati bahawa nilai perbandingan kappa (k) adalah 0.95. ELISA juga telah menunjukkan kepekaan (96.25%) dan pengkhususan (98.75%) yang tinggi berbanding dengan SNT. Oleh itu, ujian ELISA telah digunakan sebagai alternatif yang lebih praktikal untuk sero-diagnosis AD.

Untuk mengkaji sero-epidemiologi AD dalam babi di Malaysia, sejumlah 2985 sampel darah telah diambil dari seratus ladang babi dari enam buah negeri pengeluar terbesar di Malaysia dan diuji dengan ujian gE ELISA. Ladang-ladang babi ini dianggarkan mempunyai 20% dari jumlah babi di Malaysia Barat. Kajian ini telah menunjukkan bahawa 55.4% sampel darah babi dan 84% ladang babi yang dikaji mempunyai antibodi terhadap virus lapangan AD dan ini menunjukkan bahawa seroprevalen AD di Malaysia adalah tinggi sungguhpun pemvaksinan dijalankan dalam stok pembiak babi. Untuk mengurangkan prevalens AD, pemvaksinan dalam stok pengeluaran dengan vaksin yang lebih berkesan mungkin diperlukan. Disamping itu, program-program pemvaksinan dan pengurusan biosekuriti ladang yang lebih berkesan diperlukan untuk mengurangkan kadar prevalens penyakit ini sehingga penyakit ini dapat dibasmikan di Malaysia.

Pemvaksinan babi pada umur di mana tahap antibodi pasif dari ibu adalah paling rendah telah dilaporkan memberi immuniti yang optima terhadap AD. Untuk

menentukan masa yang paling sesuai bagi pemvaksinan terhadap AD, sampel darah telah diambil dari babi yang berumur empat hingga 16 minggu dari sebuah ladang AD yang endemik. Ujian SNT telah digunakan untuk mengesan antibodi AD dalam sampel darah ini dan didapati bahawa antibodi AD yang diberikan oleh ibu tidak dapat dikesan selepas umur babi yang melebihi dua belas minggu. Ini telah menunjukkan bahawa umur yang paling sesuai untuk menjalankan pemvaksinan AD pada anak babi diladang tersebut adalah pada umur minggu keempat-belas. Dalam kajian ini juga didapati bahawa anak babi yang dilahirkan oleh ibu yang telah beranak pada kali yang ketiga mempunyai tahap antibodi pasif yang lebih tinggi daripada anak babi yang dilahirkan oleh ibu dara. Ini menunjukkan bahawa pengenalpastian kerapkalian ibu beranak juga penting untuk menentukan masa pemvaksinan.

Antibodi dari ibu dilapurkan dapat mengurangkan keberkesanan pemvaksinan terhadap AD. Oleh itu, satu kajian telah dijalankan untuk mengkaji kesan antibodi ibu ke atas pemvaksinan AD dalam anak babi pada umur empat minggu. Kedua-dua vaksin hidup dan vaksin mati digunakan dalam kajian ini dan didapati bahawa antibodi dari ibu tidak mengurangkan keberkesanan pemvaksinan AD dengan ketara. Ia juga telah membuktikan bahawa dengan pemvaksinan, kerugian dalam ladang yang disebabkan oleh AD seperti kadar kematian dan tumbesaran badan yang rendah dapat dikurangkan. Kerugian ekonomi ini adalah kurang sekali dengan menggunakan vaksin hidup.

CHAPTER I

INTRODUCTION

Aujeszky's disease (AD) is a worldwide problem, causing death in many mammalian species and huge economical losses to the pig industry (OIE, 1996). The disease causes heavy mortalities among young pigs and great losses in the productivity of pig farms. It is caused by the suid herpes virus and depending on the age of the pig, the virus strain and the amount of virus infecting the animal, infection of swine could result in signs ranging from inapparent disease to death caused by infection of the respiratory tract or central nervous system (Wittman and Rziha,1989). Economic losses are mainly due to perinatal deaths, abortions, reduced fertility, reduced weight gains and respiratory problems in fattening pigs resulting in severe reduction in productivity (Gustafson, 1984).

In Malaysia, despite vaccination, sporadic outbreaks of the disease have been reported frequently in different parts of the country (Too, 1995). It has emerged to be one of the most important diseases affecting swine production and is the only other swine disease apart from classical swine fever against which vaccination is practised regularly in majority of the pig farms in Malaysia. The extent of loss in economics and productivity caused by AD in the country is still not known as no detailed study

has been conducted to measure the prevalence and impact of the disease on pig production. Therefore, a study to determine the status of the disease in the country was required.

The serum neutralization test (SNT) that is generally used in AD serological surveys has been found to be slow and laborious (Banks and Cartwright, 1983; Oirschot, 1991). The enzyme-linked immunosorbent assay (ELISA) which takes a shorter time to perform, is more sensitive and can be used for screening a large number of serum samples at a time (Durham *et al*., 1986; Oirschot *et al*, 1986). Therefore, to conduct an extensive serological study of AD in the country, a commercial gE-negative ELISA kit was used as a practical alternative to the SNT. The ELISA was evaluated using the SNT as a standard reference test. The ELISA used in this study detected antibodies directed against glycoprotein E (gE) present in the field strain of AD virus. It does not detect antibodies due to vaccination by gE negative vaccines that are used widely in the country.

To formulate a vaccination programme for the production stock in AD endemic farms, the persistence of maternal antibodies in the pigs need to be known so that the optimum time of vaccination against the disease could be determined. By conducting a vaccination programme at the proper time, maternal antibodies present in the piglets would therefore, not be able to interfere with the immune response due to vaccination. In this study, the duration of maternal protection against AD and the effect of dam parity on maternal immunity were thus determined.

It is a common practise in most Malaysian pig farms to carry out certain farm activities such as vaccination against classical swine fever (CSF) at 4-week-weaning-age. At this age, the pigs are transferred to the weaner pens and therefore, vaccination is done simultaneously with minimum effort. If vaccination against AD was also to be conducted at the same time, there would be a need to determine the effect of maternally derived antibodies on the vaccine antigens that initiate an immune response. Therefore, a study was conducted to determine the degree of interference of these antibodies in AD vaccination in 4-week-old pigs and at the same time, to compare the efficacies of the live and inactivated vaccines in the presence of these antibodies. The following values were compared between the pigs vaccinated with the live AD vaccine, an inactivated AD vaccine and control pigs: (i) body conformation (ii) respiratory signs (iii) mortality of the pigs (iv) mean rise in SNT titres of the pigs and (v) mean daily weight gain of the pigs.

Objectives of this study:

1. To compare a commercial ELISA kit and SNT for the serodiagnosis of Aujeszky's disease.
2. To determine the sero-epidemiology of AD in pigs in Malaysia.
3. To determine the persistence of passively acquired antibodies to AD in an endemic farm so that the optimum time of vaccination against the disease could be determined.
4. To determine the effect of maternally derived antibodies on vaccination against AD in four-week-old pigs. This study also assesses and compares the live and inactivated AD vaccine in improving the performance of passively immune fattening pigs in AD endemic farms.

CHAPTER II

LITERATURE REVIEW

Historical background

Aujeszky's disease (AD) was first reported in 1902 by Aladar Aujeszky when he noticed the severe disease in cattle and dog in Hungary (Aujeszky, 1902). In North America, the disease was given the name mad itch due to the severe pruritus developed in cattle. The similarities of AD with the clinical signs of rabies led some workers to name the disease pseudorabies. However, it is in swine that AD has been found to be of greatest economic significance.

Aetiology

Aujeszky's disease virus belongs to the alphaherpesvirus family (Andrewes, 1962). The pig is the natural host for AD virus, which accounts for its ability to be subclinically and latently infected (Shope, 1935; Mock *et al*., 1981). Other common farm animals infected by the virus include cattle, sheep and goats. Dogs, cats and on rare occasions, horses can also be infected (Shope, 1935). The virus replicates and produces eosinophilic intranuclear inclusion bodies in a wide variety of mammalian and avian cell cultures (Reissig and Kaplan, 1962). The cytopathic effects (CPE) of AD viruses in cell cultures are seen as bright, shining, and spheroidal (Kaplan and Vatter, 1959). The viral envelope contains at least nine structural proteins with