



**UNIVERSITI PUTRA MALAYSIA**

**CHARACTERISATION AND PATHOGENICITY STUDIES OF  
MALAYSIAN ISOLATES OF AVIAN POX VIRUS**

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**CHARACTERISATION AND PATHOGENICITY STUDIES OF  
MALAYSIAN ISOLATES OF AVIAN POX VIRUS**

**By**

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**Thesis Submitted in Fulfilment of the Requirement for  
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**DEDICATED TO**

My Beloved Mother, Late Mrs. Sivakalai Arumugam  
who passed away during the period of this study



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## LIST OF ABBREVIATIONS

CAM	Chorioallantoic membrane
CE	Chicken embryo
CEF	Chicken embryo fibroblast
CPE	Cytopathic effect
DNA	Deoxyribose Nucleic Acid
DVS	Department of Veterinary Services
EID <sub>50</sub>	Embryo infective dose 50%
EM	Electron microscopy
FP	Fowl pox
HA	Haemagglutination
Hcl	Hydrochloric acid
H & E	Haematoxylin and eosin
MAM	Ministry of Agriculture, Malaysia
MARDI	Malaysian Agricultural Research and Development Institute
NTE	Sodium choloxide Tris EDTA
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PI	Post inoculation
PTA	Phospho tungstic acid
Rf	Relative mobility factor
SDS-PAGE	Sodium dodecyl sulphate- polyacrylamide gel electrophoresis
SPF	Specific pathogen free
TCID <sub>50</sub>	Tissue culture infective dose 50%
TC-B12	Tissue culture adapted-Beaudette strain 12th passage



TEM Transmissible electron microscopy  
UPM Universiti Putra Malaysia  
VRI Veterinary Research Institute



Abstract of thesis presented to the Senate of Universiti Putra Malaysia  
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April 1997

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Avian pox is one of the important viral diseases in avian species of which, fowl pox is the most common disease and is usually controlled by vaccination. However, in recent years, outbreaks of the disease in vaccinated flocks have been reported. Therefore, it is important to study the local isolates as well as vaccine strains in order to determine the cause of the outbreaks.

This study was conducted to isolate, identify and characterise the field strains of avipox virus, which caused outbreaks among poultry in Malaysia. The isolates were also compared with the vaccine strain (TC-BI2) by pathogenicity studies in chickens. Initial characterisation of isolates involved morphology by electron microscopy, changes in embryonated chicken eggs, inclusion body formation, immunoperoxidase test and cytopathogenicity in cell culture.



Five avipox viruses were isolated from chicken, pigeon and turkey. Negative contrast electron microscopy showed the unique morphology of the isolates and did not show any differentiation among the isolates in respect to their origin. The isolates were titrated in embryonated eggs and the isolates from pigeon and turkey gave lower titre in comparison to other isolates. On chorioallantoic membrane (CAM), mother pock lesions were produced by the isolates from pigeon and turkey in comparison to the isolates from chicken which produced scattered pock lesions.

The isolates induced the formation of eosinophilic intracytoplasmic inclusion bodies in the cells of inoculated CAM. Such inclusion bodies were identified by hematoxylin-eosin staining, The indirect immunoperoxidase test was able to detect avipox virus antigens from all the isolates when fowl pox hyperimmune serum was used in this test. The isolates grew well in chicken embryo fibroblast cell culture after serial passages except for one isolate from chicken. The other isolates produced similar cytopathic effect. Titration in cell culture was also performed and the isolate from pigeon and the vaccine strain produced higher titre in comparison to the isolates from chicken and turkey.

The pathogenicity of these isolates was determined in susceptible chickens. The experiment was also conducted to evaluate field isolates for potential vaccine candidate. Another experiment was conducted to study the efficacy of tissue culture adapted vaccine, the Beaudette strain (TC-BI2). The isolates produced pock lesions at the inoculation site as well as on combs. When these isolates were given as vaccine, they showed takes in more than 80% of the chicken and they induced some protection against challenge. This showed that the isolates have potential as vaccine candidates. The tissue culture adapted Beaudette strain (TC-



B12) gave good takes in chickens. When they were challenged with the field isolates, all the chickens were 100% protected. This confirmed that TC-BI2 vaccine was highly immunogenic and an excellent vaccine.

The protein profiles of the isolates were determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis and revealed 32 polypeptide bands. All the isolates produced similar bands except for one isolate from chicken. The polypeptide bands of 35 kD and 170 kD were not observed in one isolate from chicken. This could be a "variant" strain or a new unrecognised strain.

This is the first detailed study of avipox virus isolates in Malaysia. It showed variations among the virus isolates as well as the potential as vaccine candidates. However, further studies need to be done on these variations for future work on recombinant vaccine.





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**PENCIRIAN DAN KAJIAN PATOGENISITI KE ATAS ISOLAT  
VIRUS AVIAN POX DI MALAYSIA**

Oleh

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April 1997

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Avian pox adalah salah satu penyakit virus yang penting pada spesis avian, yang mana pox ayam adalah penyakit yang kerap berlaku dan biasanya dikawal melalui pemvaksinan. Walau bagaimana pun, sejak kebelakangan ini, wabak penyakit di kalangan ayam bervaksinat telah dilaporkan. Oleh itu adalah penting untuk mengkaji isolat tempatan serta strain vaksin bagi menentukan penyebab berlakunya wabak.

Kajian ini dijalankan untuk mengisolat, mengenalpasti dan mencari strain virus Avipox lapangan yang menyebabkan wabak di kalangan ayam di Malaysia. Isolat ini juga dibandingkan dengan strain vaksin (TC-B12) melalui kajian patogenesisiti di dalam ayam. Pencirian awalan isolat melibatkan morfologi melalui



elektron mikroskop, perubahan di dalam telur ayam berembrio, pembentukan jasad rangkuman, ujian imunoperoksidase dan sitopatogenesiti di dalam sel kultur.

Lima virus avipox telah diisolat dari ayam, burung merpati dan ayam turki. Elektron mikroskop kontras negatif menunjukkan morfologi unik isolat dan tidak menunjukkan sebarang perbezaan di antara isolat-isolat walau apapun asalnya. Isolat-isolat ini dititrat di dalam telur berembrio dan isolat dari burung merpati dan ayam turki memberi titer terendah berbanding isolat lain. Di atas membran korioalantoik, lesi pok induk dihasilkan oleh isolat dari burung merpati dan ayam turki berbanding dengan isolat dari ayam yang menghasilkan lesi pok yang bertaburan.

Isolat- isolat ini mendorong pembentukan jasad rangkuman eosinofilik intrasitoplasmik di dalam sel inokulasi CAM. Jasad rangkuman ini dikenalpasti melalui stain hematoksilin- eosin. Ujian imunoperoksidase dapat mengesan antigen virus Avipox dari semua isolat apabila serum hiperimmun pox ayam digunakan. Isolat-isolat tumbuh dengan baik di dalam sel kultur fibroblas embrio ayam selepas beberapa siri laluan kecuali satu isolat dari ayam. Isolat-isolat lain menghasilkan kesan sitopatik yang serupa. Titrasi di dalam sel kultur juga dilakukan dan isolat dari burung merpati dan strain vaksin menghasilkan titer tertinggi berbanding isolat-isolat dari ayam dan ayam turki.

Patogenesiti isolat-isolat ini ditentukan ke atas ayam rentan. Ujikaji juga dijalankan bagi mengenalpasti isolat tempatan yang berpotensi sebagai calon vaksin.

Ujikaji lain dijalankan untuk mengkaji efikasi vaksin adaptasi kultur sel, strain Beaudette (TC-BI2). Isolat ini menghasilkan lesi pok ditempat inokulasi beserta dibalungnya. Apabila isolat ini diguna sebagai vaksin, ia menunjukkan pengambilan lebih 80% pada ayam dan memberi perlindungan pada cabaran. Ini menunjukkan isolat ini mempunyai potensi sebagai calon vaksin. Adaptasi kultur tisu Strain Beaudette (TC-BI2) memberi pengambilan yang baik pada ayam. Apabila mereka dicabar dengan isolat tempatan, kesemua ayam dilindungi 100%. Ini mengesahkan vaksin TC-BI2 mempunyai ciri imunogen yang tinggi dan adalah vaksin terbaik.

Profil protin isolat ditentukan dengan elektroforesis gel natrium dodesil sulfat poliakrilamida dan menghasilkan 32 band polipeptid. Kesemua isolat menghasilkan band yang serupa kecuali satu isolat dari ayam. Band polipeptid dari 35 kD dan 170 kD tidak terdapat pada satu isolat dari ayam. Ini mungkin strain "varian" atau strain baru yang belum dikenalpasti.

Ini adalah kajian isolat virus avipox terperinci yang pertama di Malaysia. Ia menunjukkan kepelbagaian dikalangan isolat- isolat virus dan mempunyai potensi sebagai calon vaksin. Walaubagaimana pun, kajian lanjutan perlu dilakukan keatas kepelbagaian ini untuk penggunaan dalam vaksin rekombinan pada masa akan datang.

## CHAPTER I

### INTRODUCTION

The poultry industry is the main component of the Malaysian livestock industry as it fetches around 70% of the total farm gate value of all local livestock production. The modern poultry industry developed from traditional backyard subsistence poultry farming through the last four decades. The traditional village level of small holder operation is also an important contributor to the production of table birds and eggs. The greater industrialisation of the industry was achieved through the policy of investment incentives by the Government in the eighties.

Malaysia achieved the present day poultry production which is around 600,000 metric tons poultry meat and 6000 million eggs annually, through several factors such as disease control, liberal policy, tariff protection, structural changes and consumers preference irrespective of their religion (Ramlah, 1993; Seri Masran, 1996).

Due to the global nature of the poultry industry and its business, it is always liable to get new type of disease causative agents from the outside or there would be an emerging new or old disease agent due to genetic variance. Therefore, poultry diseases still threaten the poultry industry. Among them is fowl pox, one of the important avian pox diseases in this region (Aini, 1990).



According to the earliest references (Lim, 1994), fowl pox disease was commonly observed in Malaysia and it was one of the major problems among village chicken. Advice on good management and proper disposal of diseased birds were used to control the disease but it was found to be ineffective. The preparation of crude vaccine from dried ground scabs in glycerol saline was tried in 1935 and even though it was safe to use, it was found to be not very effective (Lim, 1994). In 1936, pigeon pox vaccine from India was subjected to experimental infectivity test as another early attempt to control the disease. After the start of second world war, fowl pox disease was prevalent in most of the states in Malaysia and caused high mortality, up to 80% among the affected birds. Chick embryo (CE) fowl pox vaccine seed (Beaudette strain) was introduced from Weybridge, England in 1953. The extensive CE fowl pox vaccination resulted in very effective control of the disease beginning in 1957. From then onward, few isolated cases were reported (Lim, 1994).

Among domestic birds, this disease has been recognised in chicken, turkey and pigeon since the earliest days of poultry farming. In chicken, the disease is known as “fowl pox” (FP). It affects susceptible chicken of all ages, both sexes and all breeds (Tripathy,1984). Other names of this disease are bird pox, bird pox diphtheria, avian diphtheria, avian molluscum, contagious epithelioma, sorehead and canker (Cunningham,1965).

FP is endemic in Malaysia according to the Animal Health Status Report, Office International Epizootics, Regional Commission for Asia,(1985). Although this disease has been controlled by routine vaccinations, in recent years nearly 70% of the outbreaks were in vaccinated flocks ( Lim, 1994).



Since 1957, the fowl pox vaccine, Beaudette strain, was produced by Veterinary Research Institute (VRI) in chick embryo as wet live form. Imported vaccines are also available in Malaysia. Presently, a local vaccine company produces tissue culture adapted vaccine. The vaccine strain, Beaudette strain, adapted to primary chicken embryo fibroblast tissue culture was used as master seed after 12 serial passages (Aini et al., 1994). The vaccine induced high level of serum neutralising antibodies after 14 days post vaccination and it is cheaper to produce compared to chicken embryo vaccine. In addition, four polyvalent and 11 monovalent commercial fowl pox vaccine products are imported into Malaysia (DVS,1993).

Again, the disease gets significantly important due to outbreaks among vaccinated flocks and high number of cases are also reported from non-vaccinated flock (Lim,1994). Detailed study has not been undertaken to find out the reasons for the outbreaks. Fatunmbi and Reed (1996) also reported that the incidence of pox was high among chickens from previously vaccinated flock. The pox disease is important among pigeon and turkey also (Loganathan et al.,1985; Aini and Ibrahim, 1986; Lim et al.,1986)

Cross infections of various pox isolates from other species of birds may be one of the many reasons for causing such situation. This creates the necessity for detailed study of various isolates from those outbreaks. In Malaysia, pigeon farms and pigeon fanciers are increasing and outbreaks of pigeon pox with severe lesions have been reported (Loganathan et al., 1985). Therefore, there is also a need to study the pox virus isolated from pigeons, so that a suitable vaccine against pox disease can be developed in order to prevent severe pox outbreaks among

pigeons. Even turkeys, which are considered as one of the lesser species in Malaysia (Seri Masran, 1996), are susceptible and pox disease among them is very important due to prolonged generalised infection causing emaciation. Inter-species cross infection among poultry has also been reported (Aini and Ibrahim, 1986). Hampson (1989) reported that an outbreak of pox among chickens in America was controlled by pigeon pox vaccine.

Tissue culture vaccines and chicken embryo vaccines are the two types of attenuated live virus vaccines used for immunisations against avian pox. Tissue culture vaccines are more economical and more uniform than conventional chicken embryo vaccines. Tissue culture vaccines can be used in chicks as early as one-day old without causing any side effects (Tripathy, 1991).

Fowl pox virus is also important as a vector for other vaccines because this virus is able to accommodate significant amount of foreign gene while maintaining its infectivity and immunogenicity. Foreign genes responsible for specific antigens can be identified from the genes of pathogens of the chicken and can be successfully inserted into the gene of any avian pox virus. Therefore, it is also necessary to study about some aspects of biological characteristics of various local isolates of avian pox viruses for future selection of vaccine strains and subsequently to be used for recombinant vaccine production.

In recent time, many outbreaks of fowl pox and pigeon pox are confirmed at the laboratories annually from the reported cases from various parts of the country.

In the field, further pox cases are reported and noticed among other various disease outbreaks (Lim, 1994).

Therefore, the objectives of the study are:

1. to characterise the avian pox virus field isolates by conventional and molecular methods,
2. to determine the pathogenicity of avian pox virus field virus isolates *in vitro* and *in vivo*, and
3. to study the protection given by vaccinated birds against avian pox field isolates.



## CHAPTER II

### LITERATURE REVIEW

#### Background

Avian pox is not only one of the oldest known viral disease but also a serious disease that occurred world-wide for centuries (Fenner et al., 1993). The history of pox viruses has been dominated by small pox which was pandemic among human over 2000 years (Fenner, 1985). Avian pox has also been observed in avian species from time immemorial (Cunningham, 1965). The term "fowl pox" was first named for all pox diseases of birds (Tripathy, 1984).

#### Avian Pox Virus

##### Classification

Avian pox viruses affecting birds are classified as members of the genus *Avipox* virus of the subfamily *Chordopoxvirinae*, which belongs to the family *Poxviridae* according to the International Committee on Taxonomy of Viruses (I.C.T.V) (Matthews, 1982). This genus includes all members causing pox diseases among birds. The viruses have been generally named according to bird species such as pigeon, turkey, canary, starling, junco, quail, psittacine, mynah and sparrow. They share a strong serological relationship due to the nucleoprotein precipitinogen within the pox virus group (Woodroffe and Fenner, 1962 ; Mathew, 1975).

