UNIVERSITI PUTRA MALAYSIA

COMPARATIVE IN VITRO AND IN VIVO PATHOGENESIS OF EXPERIMENTALLY INDUCED INCLUSION BODY DISEASE OF BOIDS

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FPV 2013 25

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2013

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By

OMAR EMAD IBRAHIM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,

In Fulfilment of the Requirements for the Degree of Doctor of Philosophy

March 2013

IN THE NAME OF ALLAH THE MOST GRACIOUS AND MERCIFUL

DEDICATION WITH LOVE AND GRATITUDE TO:

MY PARENTS, WHO MADE IT POSSIBLE;

MY BELOVED WIFE, WHO MADE THIS WRITING ENDURABLE;

AND TO RAND, AHMAD, AL-HAMZA AND AL-FAROUK, WHO MADE IT ALL WORTHWHILE

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

COMPARATIVE *IN VITRO* AND *IN VIVO* PATHOGENESIS OF EXPERIMENTALLY INDUCED INCLUSION BODY DISEASE OF BOIDS

By

OMAR EMAD IBRAHIM

March 2013

Chair: Professor Noordin Mohamed Mustapha, PhD

Faculty: Veterinary Medicine

The inclusion body disease (IBD) is an infectious fatal disease of boid snakes characterised by behavioral abnormalities, wasting and secondary infections. Microscopically, the disease is identified by the presence of large eosinophilic cytoplasmic inclusions in multiple tissues and thus giving rise to the name of the disease.

To date, no exact agent has been conclusively incriminated as the cause of the inclusion body disease (IBD). A total of forty-eight boa and python snakes suspected of (IBD), (15) boa (33) python cases were submitted for necropsy to the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia from March 2008 to June 2009 from a recently officiated snake park located 50 kilometres south of UPM.

Using a cell culture and *in vivo* approach to search for the aetiological agent, identification and *de novo* assembled the cytopathic effects and the morphology and size of two viruses related to small round viruses the size of around 29.5 – 36 nanometer (nm). A continuous

Vero cell line was established and used to propagate and isolate the viruses in culture. In total, small round viruses were detected in 20 out of 30 suspected cases of IBD. These viruses have a typical small round viruses organization but were highly divergent. The result of virus clarification showed a visible opaque band, of the purified virus at the 30% interface while the negatively stained particles under electron microscopy showed spherical with icosahedral symmetry viral particles sized between 29.5 – 36.5 nm. More importantly, the two viral isolates, CPE, band, size and morphology were similar for both the boa and python. The presence of small round viruses out of mammals reveals that these viruses infect an unexpectedly broad range of species and represent a new reservoir of potential human pathogens.

The findings suggest that IBD is a multisystemic viral infection based on the histopathological findings in the natural cases of IBD in boa and python. In short, it suggests that the incriminating virus or agent is definitely not associated with popularly believed Type-C retrovirus.

Twenty five female BALB/c mice (6 – 8 weeks of age) were used to study the pathogenesis apart from verifying Koch's postulate of IBD via inoculation of boa and python isolates in a murine model. The findings demonstrated the ability of IBD virus from both boa and python to induce an acute and chronic infection in mice.

In conclusion, this is the first detailed study on isolated IBD virus in an attempt to adapt the viruses *in vitro* and assessing their pathogenic potential in a murine model.

Key words: IBD, boa, python, virus, mice

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERBANDINGAN IN VITRO DAN IN VIVO PATOGENESIS DARI UJI KAJI MENDORONG PENYAKIT MEMASUKKAN BADAN DARI BOIDS

Oleh

OMAR EMAD IBRAHIM

Mac 2013

Pengerusi: Profesor Noordin Mohamed Mustapha, PhD

Fakulti: Perubatan Veterinar

Penyakit jasad rangkuman (IBD) merupakan penyakit berjangkit dan merbahaya pada ular boid yang bercirikan perubahan perilaku, kurus-kering dan jangkitan sekunder.Secara mikroskopi, penyakit ini disahkan dengan kehadiran rangkuman besar serta bereosinofil pada sitoplasma pelbagai tisu dan dari sinilah nama penyakit tersebut diambil.

Sehingga kini, tiada agen khusus telah dikenalpasti secara tepat sebagai penyebab penyakit ini. Masing-masing sebanyak 48 dan 33 karkas dari boa dan ular sawa yang diterima untuk nekropsi di Jabatan Patologi dan Mikrobiologi Veterinar, Fakulti Perubatan Veterinar, Universiti Putra Malaysia digunakan dalam kajian ini. Kesemua kes diterima mulai Mac 2008 - Jun 2009 dari sebuah taman ular yang baru dirasmikan terletak 50 km selatan dari UPM.

Dengan menggunakan kultur sel dan pendekatan *in vivo* dalam mencari etiologi membawa kepada pengenalpastian dan himpunan *de novo* kesan sitopati dan morfologi serta kedua-dua

virus bulat kecil bersaiz 29.5-35 nm yang terlibat. Garisan sel Vero berterusan yang dikukuhkan telah digunakan untuk menjana dan mengasing virus dalam kultur. Secara keseluruhan, virus dikesan pada 20 dari 30 kes disyaki IBD. Virus ini mempunyai himpunan virus kecil bulat tetapi amat berlainan. Hasil dari pengasingan virus menunjukkan garisan legap dari permukaan 30% virus tertulin manakala zarah yang diwarnakan secara negatif di bawah mikroskop elektron menunjukkan zarah virus simetri ikosahedron bersaiz antara 29.5-36.5nm. Lebih penting lagi, kedua-kuda isolat virus, CPE, garisan, saiz dan morfologi adalah sama bagi boa dan ular sawa. Kehadiran virus bulat kecil ini di luar mamalia menandakan bahawa virus ini boleh menjangkiti julat sepsis yang luas dan mewakili tabungan potensi patogen manusia.

Penemuan ini mencadangkan bahawa IBD ialah jangkitan virus multisistem berdasarkan pada hasil histopatologi pada kes semulajadi IBD pada boa dan ular sawa. Pendekata,it mencadangkan bahawa virus atau agen terlibat tidak berkait dengan kepercayaan popular disebabkan oleh retrovirus Jenis C.

Dua puluh lima ekor mencit BALB/c betina berusia 6-8 minggu digunakan untuk kajian pathogenesis selain dari mengesahkan postulat Koch, dengan inokulat pada isolat boa dan ular sawa pada model murin. Penemuan menunjukkan keupayan virus IBD daripada boa dan ular sawa untuk mengaruh jangkitan akut dan kronik pada mencit.

Sebagai rumusan, ini merupakan kajian terperinci pengaisngan virus IBD dalam usaha untuk menyesuaikan virus *in vitro* dan menilai potensi kepatogenan pada model murin.

Kata kunci: IBD, boa, ular sawa, virus, mencit

ACKNOWLEDGEMENTS

At the time of completing this thesis, I would like to take this opportunity to express my gratefulness to the Almighty ALLAH, lord of all creations, who gives me strength, courage, inspiration and love to be able to go through all the days of my life and also afforded me great understanding and wisdom to complete my thesis, all the glory to his name.

I want to pause long enough to shine the spotlight on the people who helped me along the way, if only for a moment. They deserve it.

First and foremost I offer my sincerest gratitude to my supervisor Professor Dr. Noordin Mohamed Mustapha for giving me the opportunity to complete my PhD, his help and guidance throughout my graduate program. He is very understanding, caring, supportive and a great person to work with. I had a tremendous learning experience as a graduate student because I was able to study and grow under his mentorship and guidance. His constructive suggestions, criticisms and provoking have been most valuable. I am forever indebted for his kindness, patience and motivation.

I wish to express my faithful gratitude and sincere thanks to my committee members, Prof. Dato' Dr. Sheikh-Omar Abdul Rahman who filled an important place in my life as a mentor of spiritual values in this work. I would also like to express my heartfelt thanks and appreciation to Professor Dr. Zuki Abu Bakar Zakaria for his constructive supports which were really helpful towards the completion of my study.

This work would not have been possible without the support and helping hand from the staff members of the virology laboratory, in particular to Mr. Mohd Kamarudin Awang, Mr. Mohd Nazri Abd Hamid, and Mr. Shahrudin Uda Ibrahim.

I am also extremely grateful to all the staff in Microscopy Unit and Molecular Biomedicine of Institute of Bioscience, particular, Mr. Rafizu Zaman Haron, Mrs. Aminah Jusoh, Mrs. Faridah Akmal, Dr. Tan Sheau Wei and Mrs. Nancy Liew Woan Charn.

I would like to offer my special thanks and I am forever grateful to my best friend, Dr. Amer Khazaal Salih Al-Azawy for his assistance. Thank you for all the kind help.

My thanks also go to Dr. Aqil Mohammad Daher, Dr. Hamid Al-Tammemi, Dr. Ajwad Awad, Dr. Karim, Dr. Latif, Dr. Nabeel, Dr. Ibrahim, Dr. Majed Hamed, Dr. Mayada Hasson, Dr. Nathera Mohamed, Dr. Faruk Bandi, Dr. Saeed Sharif, Dr. Faiz Fawzi, Dr. Hemen Othman and Dr. Abdul Rahman for their cooperation.

My sincere thanks also to the Malaysian Government and Universiti Putra Malaysia in particular for supporting me throughout the course of my study.

I wish to express my deepest and heartfelt appreciation to my father Prof. Dr. IMAD and my greatest supporter my mother. I also would love to thank my beloved wife, my daughter RAND, and my sons AHMAD, ALHAMZA and ALFAROUK. I am also thankful to my sister SURA and my brother ABDULLAH also to my uncle Dr. TARIK for being supportive, helpful throughout my study. Without my family, I would never be able to accomplish this challenging task.

Finally, many thanks to all who have helped or contributed in one way or other towards the completion of this study.

I certify that a Thesis Examination Committee has met on 01 March 2013 to conduct the final examination of Omar Emad Ibrahim on his thesis entitled "Comparative *in Vitro* and *in Vivo* Pathogenesis of Experimentally Induced Inclusion Body Disease of Boids" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy**.** The members of the Supervisory Committee were as follows:

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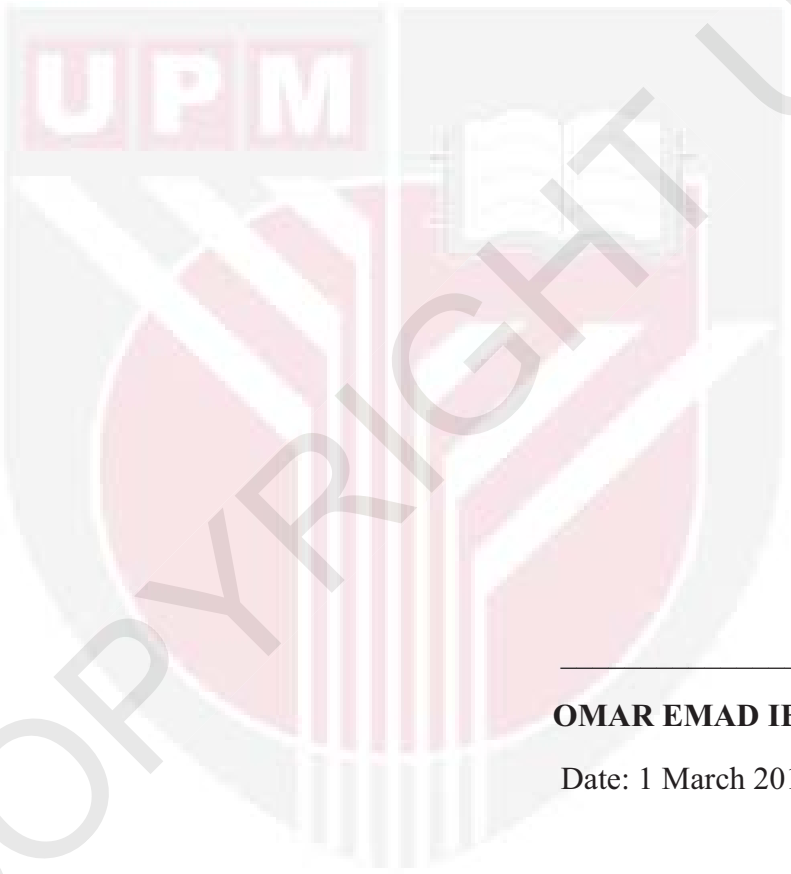
DECLARATION

I declare that the thesis is my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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OMAR EMAD IBRAHIM

Date: 1 March 2013

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CHAPTER 1

GENERAL INTRODUCTION

Inclusion Body Disease (IBD) is the leading cause of death in large non-venomous captive snakes of the Boidae and Pythonidae family. It is a common disorder characterised by the formation of eosinophilic intracytoplasmic inclusion bodies in the epithelial cells of major organs (Schumacher, Jacobson, Homer, & Gaskin, 1994).The intracytoplasmic inclusion bodies in boa constrictor were mainly disseminated in the visceral organs, however the inclusions in python most often localised in the brain. Similarly, the development of the disease in boa constrictor takes much more compared to pythons.

In recent years, there has been an increasing interest in the understanding of the disease aetiology, pathogenesis, route of transmission, epidemiology and treatment (Vancraeynest *et al.*, 2006). Chronologically, the past thirty years showed a high incidence of IBD in captive Burmese python (late seventies extending into the mid eighties), while in the early nineties more cases were diagnosed in boa constrictors (Schumacher *et al.*, 1994).

Considerable amount of literature on IBD showed the relationship between the C-type retrovirus like particles albeit never been proven via Koch's postulate (Schumacher *et al*., 1994, Jacobson *et al*., 2001). Thus, until today the agent, route of entry, cross species infection and pathogenesis of IBD still remains as an enigma both in boa and python. We hypothesized that "pathogenesis of inclusion body disease is not associated with type C retrovirus infection"

As such, issues involve in the aetiology and pathogenesis associated with the agents causing IBD between python and boa need to be elucidated.

Therefore the objectives for this study were to:

1. elucidate the pathological changes of IBD in captive boa and python
2. isolate and identify the etiological agents of IBD
3. investigate the *in vitro* pathogenesis of IBD associated with the agents isolated from boa and python
4. investigate the *in vivo* pathogenesis of the disease associated with the virus from boa and python in mouse model

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