UNIVERSITI PUTRA MALAYSIA

DETECTION OF PORCINE BOCAVIRUS IN MALAYSIAN SWINE HERDS

DANIEL MOHAN JACOB

FPV 2015 22

DETECTION OF PORCINE BOCAVIRUS IN MALAYSIAN SWINE HERDS

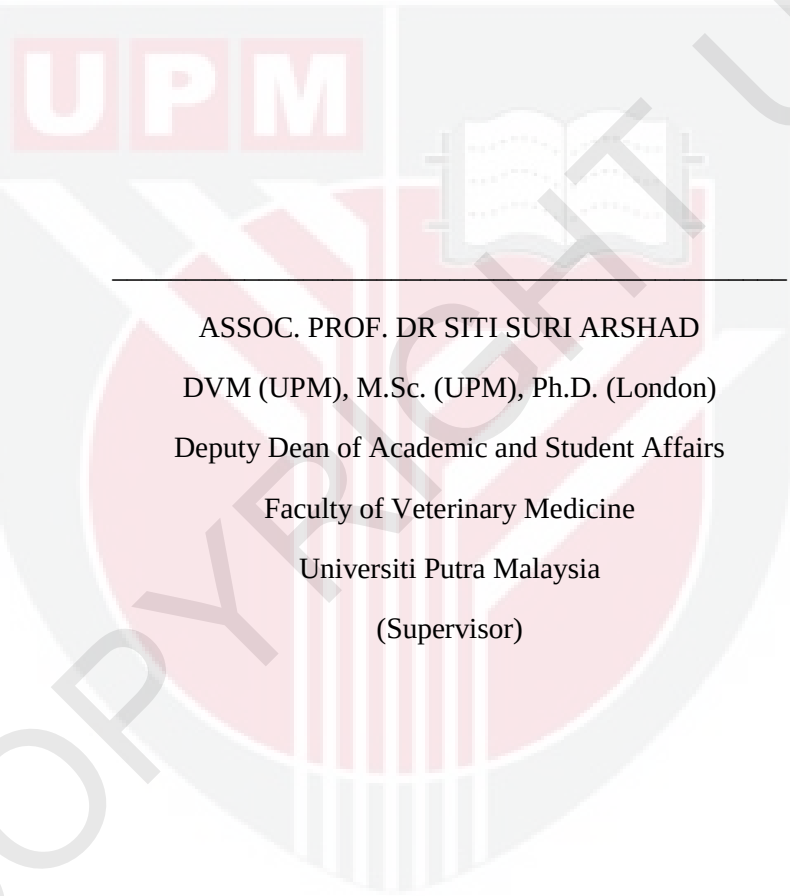
DANIEL MOHAN JACOB

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia,
Serdang, Selangor Darul Ehsan.

MARCH 2015

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Detection of Porcine Bocavirus in Malaysian Swine Herds", by Daniel Mohan Jacob and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project



_____

ASSOC. PROF. DR SITI SURI ARSHAD

DVM (UPM), M.Sc. (UPM), Ph.D. (London)

Deputy Dean of Academic and Student Affairs

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

_____

DR OOI PECK TOUNG

DVM (UPM), Ph.D. (Glasgow)

Senior lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DR GAYATHRI THEVI SELVARAJAH

DVM (UPM), Ph.D. (Netherlands)

Senior lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DEDICATIONS

This project paper is dedicated to the One Almighty God, who had created me and made all things possible,

To my family,

Grandmother

Father

Mother

Brother, Sister

Vivian Tan

& my late mother

And to all my teachers who have committed themselves towards the noble cause of education.

ACKNOWLEDGEMENTS

It is with deepest appreciation and gratitude that I thank God and all those who have made this project paper a reality.

To the persons that have assisted my throughout this project, I would firstly like to thank my project supervisor, Assoc. Prof. Dr Siti Suri Arshad for the time, wisdom, expertise, and guidance that she had granted me throughout the duration of this project, and my studies at the faculty.

To my co-supervisors, Dr Ooi Peck Toung and Dr Gayathri Thevi Selvarajah for their unwavering support and encouragement to improve the project, and myself personally. Special thanks to Dr Faruku Bande for his assistance for without it, this project paper would not exist.

I would also like to thank the post-graduate students and staff of the Virology Lab, UPM which includes En. Din, Krishnan, and Ashwaq for always lending me a helping hand when I needed it, and sharing good company.

A special thank you to all my classmates of DVM 2015 who assisted my directly or indirectly in this project with special mention to Kuiek Ah Meng, Sia Bang Wen, Tan Yi Wei, and Chai Ai Ting.

Last but not least, my most heartfelt gratitude to my family; my grandmother, father, mother, brother and dear sister for their love and support throughout my studies. Not forgetting as well, my significant other, Vivian Tan.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
µl	Microliter
µM	Micromolar
°C	Degree Celsius
ARD	Acute Respiratory Disease
ATPase	Adenosine triphosphatase
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
BPV	Bovine bocavirus
Csl BoV	California sea lion bocavirus
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxyribonucleotide triphosphate
EtBr	Ethidium bromide
FBoV	Feline bocavirus
g	Gram
GBoV	Gorilla bocavirus
HBoV	Human bocavirus
HK	Hong Kong
ICTV	International Committee on Taxonomy of Viruses
ILN	Inguinal lymph node
KDN	Kidney
mA	Milliampere
$MgCl_2$	Magnesium chloride
min	Minutes
ml	Milliliter

MLN	Mesenteric lymph node
MVC	Minute virus of canines
NCBI	National Centre for Biotechnology Information
ng	Nanogram
no.	Number
NP1	Nuclear phosphoprotein
NS1	Nonstructural protein 1
NTC	No template control
ORF	Open reading frame
PBoV	Porcine bocavirus
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PCV2	Porcine circovirus type-2
Pg	Pig
PMWS	Post Weaning Multisystemic Wasting Syndrome
PRRSV	Porcine reproductive and respiratory syndrome virus
PTTV2	Porcine torque teno virus species 2
RTU	Ready-to-use
SPL	Spleen
sPLA	Secretory phospholipase
TAE	Tris-acetate-EDTA
UK	United Kingdom
USA	United States of America
V	Volt
v	Version
VP1	Viral protein 1
VP2	Viral protein 2
x g	Relative centrifugal force

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

PENGESANAN PORSIN BOCAVIRUS DALAN KAWANAN PORSIN MALAYSIA

Oleh

Daniel Mohan Jacob

2015

Penyelia: Assoc. Prof. Dr Siti Suri Arshad

Penyelia bersama:

Dr Ooi Peck Toung

Dr Gayathri Thevi Selvarajah

Sejak penemuan porsin bocavirus (PBoV) di Sweden pada tahun 2009, sebanyak 11 negara lain telah melaporkan. Oleh sebab Malaysia mempunyai industri ternakan porsin yang signifikan, projek ini bermatlamat untuk mengesan PBoV dalam kawanan porsin tempatan. Menggunakan teknik persampelan mudah, 11 ekor porsin telah disampel dari tiga buah ladang yang terletak di negeri Perak dan Selangor. Setiap sampel tisu yang diperoleh daripada pembedahan karkas telah diuji dengan kaedah PCR konvensional menggunakan primer spesifik yang direka untuk menyasar gen *nonstructural* (NS) 1. Berdasarkan keputusan PCR, 10 daripada 11 porsin positif untuk PBoV. Untuk

pengesahan lanjut, penjujukan nukleotid NS1 telah dilakukan, dan jujukannya dibandingkan dengan pencilan rujukan. Tambahan pula, analisa filogenetik telah dilakukan untuk mengenal pasti hubungan antara strain Malaysia dengan pencilan rujukan. Analisa bioinformatik menunjukkan bahawa pencilan Malaysia hampir sama dengan PBoV3 dari USA. Untuk lebih memahami PBoV di Malaysia, kajian prevalens, pengasingan virus, dan penjujukan genom lengkap perlu dilaksanakan. Kesimpulannya, negara Malaysia merupakan negara ketiga belas mengesan PBoV dalan kawanan porsinnya melaui kaedah PCR dan penjujukan separa gen NS1.

Kata kunci: Porsin bocavirus, PCR, penjujukan, porsin, analisa filogenetik

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 - Project

DETECTION OF PORCINE BOCAVIRUS IN MALAYSIAN SWINE HERDS

By

Daniel Mohan Jacob

2015

Supervisor: Assoc. Prof. Dr Siti Suri Arshad

Co-supervisors:

Dr Ooi Peck Toung

Dr Gayathri Thevi Selvarajah

Porcine bocavirus (PBoV), which was first discovered in Swedish pigs in 2009, has since been detected in 11 other countries at the time of writing. As Malaysia is host to a significant swine livestock industry, this study aimed to describe PBoV in local swine herds. Using the convenience sampling method, 11 pigs were sampled from three farms located in the states of Perak and Selangor. Each tissue sample obtained from necropsy were subjected to conventional PCR assay using specific primers designed to target the conserved nonstructural protein 1 (NS1) gene. Based on PCR assay, 10 out of 11 pigs were positive for PBoV. For further confirmation, partial nucleotide sequencing of the NS1 gene was performed, and the sequence compared to reference isolates. In addition,

phylogenetic analysis was performed to determine the genetic relationship of Malaysian PBoV strains to reference isolates. Bioinformatics analysis determined that the Malaysian strain was highly similar (95%) to PBoV3 isolated in USA. To further understand PBoV in Malaysia, prevalence study, virus isolation and complete genome sequencing should be performed. In conclusion, Malaysia is the 13th country in the world that has described PBoV in its swine herds by PCR assay and partial sequencing of the NS1 gene.

Key words: Porcine bocavirus, PCR, sequencing, swine, phylogenetic analysis

1.0 INTRODUCTION

1.1 *Bocavirus*

Bocavirus is a genus of the family *Parvoviridae*, subfamily *Parvovirinae* which has been recognized in veterinary medicine since the early 1960's. To date, bocaviruses which have been discovered include bovine parvovirus (BPV) (Chen *et al*., 1986), minute virus of canine (MVC) (Binn *et al*., 1970), porcine bocavirus (PBoV) (Blomström *et al*., 2009), gorilla bocavirus (GBoV) (Kapoor *et al*., 2010a), feline bocavirus (FBoV) (Lau *et al*., 2012), and California sea lion bocavirus (Csl BoV) (Li *et al*., 2011). As studies continue to be carried out, it is possible that bocaviruses will be detected in more animal species.

Bocaviruses have been of increasing importance in recent years as studies have shown human infections with human bocavirus (HBoV), which may have had a zoonotic origin (Schildgen *et al*., 2012). HBoV was first identified in Sweden from nasopharyngeal aspirate samples in children with respiratory infections (Allander *et al*., 2005). Since then, it has been associated with acute lower respiratory tract disease in children (Fry *et al*., 2007), and adults, especially in elderly patients (Liu *et al*., 2011). In addition, HBoV has also been detected from stool samples in children with gastroenteritis (Arthur *et al*., 2009).

In Malaysia, HBoV was first detected in a 13-month old boy with pneumonia and underlying asthma (Etemadi *et al*., 2012). Subsequently, a study of 125 hospitalized children with Acute Respiratory Disease (ARD) were analyzed for the presence of HBoV. The study showed that 5.6% (7/125) of the cases were positive for HBoV (Tg Rogayah *et al*., 2014).

Because HBoV and PBoV both belong to the genus *Bocavirus* and family *Parvoviridae*, they share many similarities in terms of their virus characteristics. At the amino acid sequence level, the predicted nonstructural protein 1 (NS1) gene of PBoV exhibited sequence identities of 38.0 – 54.7% to HBoV (Zeng *et al*., 2011). Hence, experience from HBoV research may be applied to PBoV, especially since there is an increasing number of studies involving the pathogenicity of HBoV.

1.2 Porcine Bocavirus

Porcine bocavirus (PBoV) is a recently discovered virus that infects pigs and is classified within the *Bocavirus* genus (family *Parvoviridae*, subfamily *Parvovirinae*). It was first discovered in 2009 in Sweden, when a novel porcine parvovirus with genetic relationship to bocaviruses was incidentally found in pigs with post-weaning multisystemic wasting syndrome (PMWS) (Blomström *et al*., 2009). Since its discovery, PBoV has been detected in 12 countries. Those countries are Sweden, China, USA, Canada, Mexico, Romania, Hungary, Uganda, Korea, Cameroon, UK (Zhou *et al*., 2014), and Thailand (Saekhow & Ikeda, 2014).

As more countries detect PBoV, more strains are being discovered. At the time of writing, nine genotypes of PBoV had been identified and sequenced. However, an agreeable method of classifying these strains has yet to be established. Currently, the two methods of classification that are widely accepted are based on the sequencing of the NS1 and viral protein 1 gene (VP1) of PBoV (Zhou *et al*., 2014). Based on these methods of classification, PBoV's are named based on their order of discovery and subgroup. Presently, known genotypes of PBoV's are: PBoV1, PBoV2, PBoV3, PBoV4, PBoV5, PBoV3C, PBoV-6V, PBoV-7V, and swBoV CH437.

To date, the significance of PBoV in pig production, pig health, and public health has yet to be determined. Studies have shown that the detection rate of PBoV is significantly higher in sick pigs compared to healthy pigs. Moreover, positive samples for PBoV are also significantly higher in pigs co-infected with porcine circovirus type-2 (PCV2), porcine reproductive and respiratory syndrome virus (PRRSV), and porcine torque teno virus species 2 (PTTV2), thus suggesting that PBoV may be involved with PMWS in post-weaning piglets (Zhai *et al*., 2010). It has also been indicated that PBoV may be associated with diarrhoea and respiratory disease. (Zhang *et al*., 2015).

Despite the significant contribution of swine farming to the Malaysian livestock industry, no study has been carried out to determine the presence of PBoV in Malaysia. Hence, this study was undertaken to fulfill the following objectives:

i. To detect the presence of porcine bocavirus in pigs in Malaysia using the conventional PCR method.

ii. To confirm PCR detection of porcine bocavirus by partial DNA sequencing of the NS1 gene.

For this research, the following hypotheses were proposed:

i. Porcine bocavirus is detected in tissue samples of local pigs by conventional PCR.

ii. Partial DNA sequence of the NS1 gene is similar to that of reference isolates.

REFERENCES

Allander, T., Tammi, M. T., Eriksson, M., Bjerkner, A., Tiveljung-Lindell, A., & Andersson, B. (2005). Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(41), 12891–12896.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*, 403–410.

Arthur, J. L., Higgins, G. D., Davidson, G. P., Givney, R. C., & Ratcliff, R. M. (2009). A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathogens*, *5*(4), e1000391.

Binn, L. N., Lazar, E. C., Eddy, G. a, & Kajima, M. (1970). Recovery and characterization of a minute virus of canines. *Infection and Immunity*, *1*(5), 503–508.

Blomström, A. L., Belák, S., Fossum, C., Fuxler, L., Wallgren, P., & Berg, M. (2010). Studies of porcine circovirus type 2, porcine boca-like virus and torque teno virus indicate the presence of multiple viral infections in postweaning multisystemic wasting syndrome pigs. *Virus Research*, *152*, 59–64.

Blomström, A.-L., Belák, S., Fossum, C., McKillen, J., Allan, G., Wallgren, P., & Berg, M. (2009). Detection of a novel porcine boca-like virus in the background of porcine circovirus type 2 induced postweaning multisystemic wasting syndrome. *Virus Research*, *146*(1-2), 125–9.

Cadar, D., Cságola, A., Lorincz, M., Tombácz, K., Kiss, T., Spînu, M., & Tuboly, T. (2011). Genetic detection and analysis of porcine bocavirus type 1 (PoBoV1) in European wild boar (Sus scrofa). *Virus Genes*, *43*, 376–379.

Carmichael, L. E., Schlafer, D. H., & Hashimoto, A. (1994). Minute virus of canines (MVC, canine parvovirus type-1): pathogenicity for pups and seroprevalence estimate. *Journal of Veterinary Diagnostic Investigation : Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, *6*, 165–174.

Chen, K. C., Shull, B. C., Moses, E. A., Lederman, M., Stout, E. R., & Bates, R. C. (1986). Complete nucleotide sequence and genome organization of bovine parvovirus. *Journal of Virology*, *60*, 1085–1097.

Cheng, W., Li, J., Huang, C., Yao, D., Liu, N., Cui, S., Jin, Y., & Duan, Z. (2010). Identification and nearly full-length genome characterization of novel porcine bocaviruses. *PloS One*, *5*(10), e13583.

Choi, M.-G., Park, S.-J., Nguyen, V.-G., Chung, H.-C., Kim, A.-R., & Park, B.-K. (2014). Molecular detection and genetic analysis of porcine bocavirus in Korean domestic swine herds. *Archives of Virology*, *159*(6), 1487–92.

Etemadi, M. R., Jalilian, F. A., Abd Wahab, N., Jahanshiri, F., Amini, R., Othman, N., & Sekawi, Z. (2012). First detected human bocavirus in a Malaysian child with pneumonia and pre-existing asthma: a case report. *The Medical Journal of Malaysia*, *67*(4), 433–4.

Fry, A. M., Lu, X., Chittaganpitch, M., Peret, T., Fischer, J., Dowell, S. F., Anderson, L. J., Erdman, D., & Olsen, S. J. (2007). Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *The Journal of Infectious Diseases*, *195*, 1038–1045.

Huang, J., Mor, S. K., Erber, J., Voss, E., & Goyal, S. M. (2014). Detection and characterization of porcine bocavirus in the United States. *Archives of Virology*, *159*(7), 1797–801.

ICTV Virus Taxonomy. (2014). Retrieved from http://ictvonline.org/virusTaxonomy.asp

Jartti, T., Hedman, K., Jartti, L., Ruuskanen, O., Allander, T., & Söderlund-Venermo, M. (2012). Human bocavirus-the first 5 years. *Reviews in Medical Virology*, *22*(1), 46-64.

Jiang, Y. H., Xiao, C. T., Yin, S. H., Gerber, P. F., Halbur, P. G., & Opriessnig, T. (2014). High prevalence and genetic diversity of porcine bocaviruses in pigs in the USA, and identification of multiple novel porcine bocaviruses. *Journal of General Virology*, *95*, 453–465.

Kapoor, A., Mehta, N., Esper, F., Poljsak-Prijatelj, M., Quan, P. L., Qaisar, N., Delwart, E., & Lipkin, W. I. (2010a). Identification and characterization of a new bocavirus species in gorillas. *PLoS ONE*, *5*, e11948.

Kapoor, A., Simmonds, P., Slikas, E., Li, L., Bodhidatta, L., Sethabutr, O., Triki, H., Bahri. O., Oderinde, B. S., Baba, M. M., Bukbuk, D. N., Besser, J., Bartkus, J., & Delwart, E. (2010b). Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *The Journal of Infectious Diseases*, *201*, 1633–1643.

Kapoor, A., Slikas, E., Simmonds, P., Chieochansin, T., Naeem, A., Shaukat, S., Alam, M. M., Sharif, S., Angez, M., Zaidi, S., & Delwart, E. (2009). A newly identified bocavirus species in human stool. *The Journal of Infectious Diseases*, *199*, 196–200.

Lau, S. K. P., Woo, P. C. Y., Yeung, H. C., Teng, J. L. L., Wu, Y., Bai, R., Fan, R. Y., Chan, K.-H., & Yuen, K.-Y. (2012). Identification and characterization of bocaviruses in cats and dogs reveals a novel feline bocavirus and a novel genetic group of canine bocavirus. *The Journal of General Virology*, *93*(Pt 7), 1573–82.

Lau, S. K. P., Woo, P. C. Y., Yip, C. C. Y., Li, K. S. M., Fu, C. T. Y., Huang, Y., Chan, K.-H., & Yuen, K.-Y. (2011). Co-existence of multiple strains of two novel porcine bocaviruses in the same pig, a previously undescribed phenomenon in members of the family Parvoviridae, and evidence for inter- and intra-host genetic diversity and recombination. *The Journal of General Virology*, *92*(Pt 9), 2047–59.

Li, B., Ma, J., Xiao, S., Fang, L., Zeng, S., Wen, L., Zhang, X., Ni, Y., Guo, R., Yu, Z., Zhou, J., Mao, A., Lv, L., Wang, X., & He, K. (2012). Complete genome sequence of a novel species of Porcine Bocavirus, PBoV5. *Journal of Virology*, *86*(2), 1286–7.

Li, B., Xiao, S., Ma, J., Liu, Y., Mao, L., Wen, L., Mao, A., Zhang, X., Ni, Y., Guo, R., Zhou, J., Yu, Z., Lv, L., Wang, X., Fang, L., Chen, H., & He, K. (2011). Development of a novel TaqMan-based real-time PCR assay for the detection of porcine boca-like virus (Pbo-likeV). *Virology Journal*, *8*(1), 357.

Li, L., Shan, T., Wang, C., Côté, C., Kolman, J., Onions, D., Guland, F. M. D., & Delwart, E. (2011). The fecal viral flora of california sea lions. *Journal of Virology*, *85*(19), 9909–9917.

Liu, M., Li, Y., Sun, D., Xia, Y., Huang, J., & Guo, L. (2014). Detection and genetic analysis of porcine bocavirus in different swine herds in North Central China. *TheScientificWorldJournal*, *2014*, 947084.

Liu, W.-K., Chen, D.-H., Liu, Q., Liang, H.-X., Yang, Z.-F., Qin, S., & Zhou, R. (2011). Detection of human bocavirus from children and adults with acute respiratory tract illness in Guangzhou, southern China. *BMC Infectious Diseases*, *11*(1), 345.

McKillen, J., McNeilly, F., Duffy, C., McMenamy, M., McNair, I., Hjertner, B., Millar, A., Mckay, K., Lagan, P., Adair, B., & Allan, G. (2011). Isolation in cell cultures and initial characterisation of two novel bocavirus species from swine in Northern Ireland. *Veterinary Microbiology*, *152*, 39–45.

Qu, X. W., Liu, W. P., Qi, Z. Y., Duan, Z. J., Zheng, L. S., Kuang, Z. Z., Zhang, W. J., & Hou, Y. De. (2008). Phospholipase A2-like activity of human bocavirus VP1 unique region. *Biochemical and Biophysical Research Communications*, *365*, 158–163.

Saekhow, P., & Ikeda, H. (2014). Prevalence and genomic characterization of Porcine Parvoviruses detected in Chiangmai area of Thailand in 2011. *Microbiology and Immunology*, *59*(2), 1–7.

Schildgen, O., Qiu, J., & Söderlund-Venermo, M. (2012). Genomic features of the human bocaviruses. *Future Virology 7*(1), 31-39.

Sun, Y., Chen, A. Y., Cheng, F., Guan, W., Johnson, F. B., & Qiu, J. (2009). Molecular characterization of infectious clones of the minute virus of canines reveals unique features of bocaviruses. *Journal of Virology*, *83*, 3956–3967.

Tg. Rogayah, T.A.R., Fauziah, M.K., Mohd Apandi, Y., Zarina, M.Z., Nur Izmawati, A.R., Nur Azrenawaty, M.N., Tg Aman Arif, T.M.A.S. & Zainah, S. (2014). Molecular epidemiology of human bocavirus in children with acute respiratory infections in Malaysia. *Innovative Journal of Medical and Health Science*, *2*, 319–323.

Yang, W. zhu, Yu, J. mei, Li, J. song, Cheng, W. xia, Huang, C. ping, & Duan, Z. jun. (2012). Genome characterization of a novel porcine bocavirus. *Archives of Virology*, *157*, 2125–2132.

Zeng, S., Wang, D., Fang, L., Ma, J., Song, T., Zhang, R., Chen, H., & Xiao, S. (2011). Complete coding sequences and phylogenetic analysis of porcine bocavirus. *Journal of General Virology*, *92*, 784–788.

Zhai, S., Yue, C., Wei, Z., Long, J., Ran, D., Lin, T., Deng, Y., Huang, L., Sun, L., Zheng, H., Gao, F., Zheng, H., Chen, S., & Yuan, S. (2010). High prevalence of a novel porcine bocavirus in weanling piglets with respiratory tract symptoms in China. *Archives of Virology*, *155*(8), 1313–7.

Zhang, Q., Zhang, C., Gao, M., He, X., Diao, Y., Goyal, S. M., Mor, S. K., & Huang, J. (2015). Evolutionary, epidemiological, demographical, and geographical dissection of porcine bocavirus in China and America. *Virus Research*, *195*, 13–24.

Zhang, Z., Zheng, Z., Luo, H., Meng, J., Li, H., Li, Q., Zhang, X., Ke, X., Bai, P., Mao, P., Hu, Q., & Wang, H. (2012). Human Bocavirus NP1 Inhibits IFN- Production by Blocking Association of IFN Regulatory Factor 3 with IFNB Promoter. *The Journal of Immunology*, *189*, 1144–1153.

Zhou, F., Sun, H., & Wang, Y. (2014). Porcine Bocavirus: Achievements in the Past Five Years. *Viruses*, *6*(12), 4946–4960.