



UNIVERSITI PUTRA MALAYSIA

DETECTION OF BOCAVIRUS IN MALAYSIAN CATS AND DOGS

LEE CHEE YIEN

FPV 2016 6

DETECTION OF BOCAVIRUS IN MALAYSIAN CATS AND DOGS

LEE CHEE YIEN

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 2016

CERTIFICATION

It is hereby certified that we have read this project paper entitled “Detection of Bocavirus in Malaysian Cats and Dogs”, by Lee Chee Yien 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), MSc. (UPM), PhD. (London)
Deputy Dean of Academic and Student Affairs
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

DR OOI PECK TOUNG
DVM (UPM), PhD. (Glasgow)
Senior lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

DR GAYATHRI THEVI SELVARAJAH

DVM (UPM), PhD. (Netherlands)

Senior lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DR NOR YASMIN ABD. RAHAMAN

DVM (UPM), PhD. (UPM)

Senior lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DEDICATIONS

This project paper is dedicated to

My family,

Grandfather

Grandmother

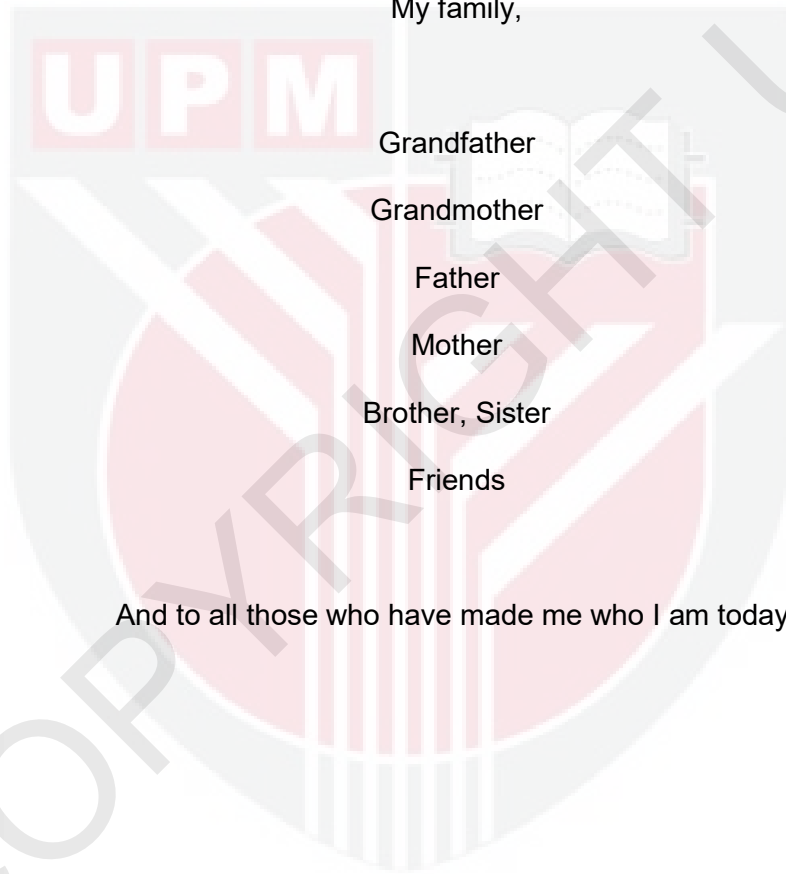
Father

Mother

Brother, Sister

Friends

And to all those who have made me who I am today.



© COPYRIGHT UPM

ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude and appreciation to Gohonzon and all those who have lend me a helping hand in making this project a success.

First and foremost, I would like to express my sincere gratitude and appreciation to my project supervisor, Assoc. Prof. Dr Siti Suri Arshad for the limitless guidance, knowledge and support throughout the duration of this project, and my years in the faculty.

In addition, million thanks to my co-supervisors, Dr Ooi Peck Toung, Dr Gayathri Thevi Selvarajah and Dr Nor Yasmin Abd. Rahaman for continuously sharing their experience and thoughts for the improvement of this project. Special thanks to Dr Daniel Mohan for he never fail to taught me everything that is crucial in completing this project.

I would also like to thank the staffs and post-graduate students of the Virology Lab and Parasitology Lab of the faculty, including En. Din, En. Rusdam, Vynter, Ashwaq, Kiven, and Mira for guiding me around the lab.

Special thanks to all of my classmates of DVM 2016 who assisted me directly or indirectly in this project, especially to Frankie Lau, Tan Shin-Yi, Chua Vi Vian and Jong Kwang Yan.

Last but not least, not forgetting my most supportive loves one, my father, mother, brother and sister for their unconditional love and support throughout my journey of life.

CONTENTS

	Page No.
Title	i
Certification	ii
Dedications	iv
Acknowledgements	v
Contents	vi
List of Tables	viii
List of Figures	ix
List of Abbreviations	xi
Abstrak	xiv
Abstract	xvi
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	4
2.1 Virus Structure	4
2.2 Taxonomy and Nomenclature	6
2.3 Epidemiology	6
2.4 Detection Methods	7
2.5 Clinical Manifestation and Pathology	8
3.0 MATERIALS AND METHODS	10
3.1 Animals	10
3.2 Sample Selection	10
3.3 Tissue Processing and Sample Pooling	11
3.4 DNA Extraction	11
3.5 Measurement of DNA Concentration	12
3.6 Primer Selection	12
3.7 Polymerase Chain Reaction	14
3.8 Agarose Gel Electrophoresis	15
3.9 DNA Purification	16
3.10 DNA Sequencing	17
3.11 Bioinformatics Analysis of Porcine Bocavirus Partial NS1 Gene Sequence	17
3.11.1 Sequence Editing and Assembly	17
3.11.2 Basic Local Alignment Search Tool (BLAST)	18
3.11.3 Multiple Alignments	18
3.11.4 Construction of Molecular Phylogenetic Tree	19
4.0 RESULTS	20
4.1 PCR Amplification	20
4.2 Bioinformatics Analysis of Malaysian FBoV Isolates	25

4.2.1	Sequence Editing and Assembly	25
4.2.2	Basic Local Alignment Search Tool (BLAST)	25
4.2.3	Multiple Alignment and Pairwise Comparison	26
4.2.4	Construction of Phylogenetic Tree	28
5.0	DISCUSSION	31
5.1	Detection of Canine Bocavirus (CBoV) in Malaysia	31
5.2	Detection of Feline Bocavirus (FBoV) in Malaysia	31
5.3	Bocavirus Detection in Mesenteric Lymph Node of Malaysian Animal Population	32
5.4	Partial Nonstructural Protein 1 (NS1) Gene Sequencing and Bioinformatics Analysis of Malaysian Isolated FBoV	33
	CONCLUSION	35
	RECOMMENDATIONS	36
	REFERENCES	37

LIST OF TABLES

	Page	
Table 3.1	Primer set for detection of bocavirus by conventional PCR assay	13
Table 3.2	Optimized cycling conditions of conventional PCR assay for detection of canine bocavirus and feline bocavirus	14
Table 3.3	Optimized cycling conditions of conventional PCR assay for detection of canine minute virus	15
Table 4.1	Sequences obtained from sequencing of the partial NS1 gene from local FBoV isolates from 2 cats with designated identifications	25
Table 4.2	Reference isolates of FBoV from Genbank®	27

LIST OF FIGURES

		Page
Figure 2.1	Comparative genome organization of FBoV, CBoV strain Con-161 and CBoV strain HK882F	5
Figure 4.1	PCR assay for pool samples for all cats using specific primers targeting the conserved NS1 gene of feline bocavirus to produce 133 bp PCR products	20
Figure 4.2	PCR assay for pool samples for dog-1 to dog-11 using specific primers targeting the conserved NS1 gene of canine bocavirus to produce 128 bp PCR products	21
Figure 4.3	PCR assay for individual tissue samples for cat-1 and cat-2 using specific primers targeting the conserved NS1 gene of canine bocavirus to produce 128 bp PCR products	22
Figure 4.4	Bar chart showing frequency of positive FBoV detection in individual tissue samples of positive cats	23
Figure 4.5	PCR assay for individual tissue samples for dog-11 using specific primers targeting the conserved NS1 gene of canine bocavirus to produce 128 bp PCR products	24
Figure 4.6	Bar chart showing frequency of positive CBoV detection in individual tissue samples of positive dogs	24
Figure 4.7	Partial NS1 sequence of FBoV_Malaysia_C1 with length 110 bp	26
Figure 4.8	Partial NS1 sequence of FBoV_Malaysia_C2 with length 101 bp	26
Figure 4.9	Sequence identity matrix with pairwise comparison to compare sequence identity of the two nucleotides derived from the partial NS1 gene of local FBoV isolates with reference isolates	27

- Figure 4.10 Sequence identity matrix with pairwise comparison to compare sequence identity of the two nucleotides derived from the partial NS1 gene of local FBoV isolates with five local PBoV isolates 28
- Figure 4.11 Unrooted phylogenetic tree of Malaysian FBoV and reference isolates 30

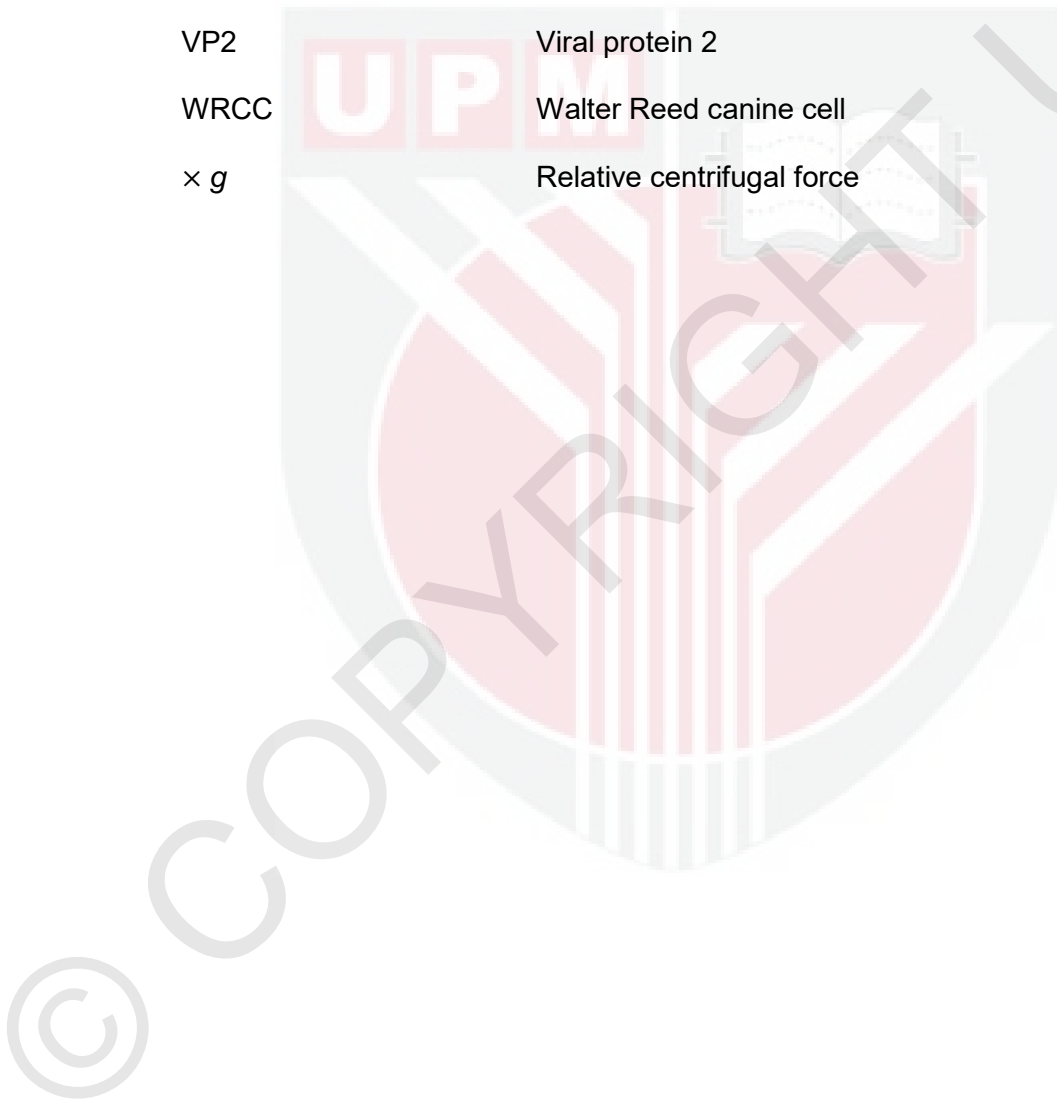


LIST OF ABBREVIATIONS

%	Percent
µl	Microliter
µM	Micromolar
°C	Degree Celsius
aa	Amino acid
ATPase	Adenosine triphosphatase
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
BPV	Bovine parvovirus
CBoV	Canine bocavirus
CBoV2	Canine bocavirus 2
CBoV3	Canine bocavirus 3
CMV	Canine minute virus
Csl BoV	California sea lion bocavirus
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FBoV	Feline bocavirus
FBoV2	Feline bocavirus 2
g	Gram
GBoV	Gorilla bocavirus
HBoV	Human bocavirus

HI	Hemagglutination-inhibition
IACUC	Institutional Animal Care and Use Committee
ICTV	International Committee on Taxonomy of Viruses
kbp	Kilobase pairs
mg	Milligram
mg/kg	Milligram per kilogram
MgCl ₂	Magnesium chloride
min	Minutes
ml	Milliliter
mm	Millimeter
mM	Millimolar
MVC	Minute virus of canine
NCBI	National Center for Biotechnology Information
NJ	Neighbour-joining
NP1	Nuclear phosphoprotein
NS1	Nonstructural protein
nt	Nucleotide
NTC	No template control
ORF	Open reading frame
PBoV	Porcine bocavirus
PCR	Polymerase Chain Reaction
RNase	Ribonuclease
TAE	Tris-acetate-ethylenediaminetetraacetic acid
U	Unit
UPM	Universiti Putra Malaysia

USA	United States of America
UV	Ultraviolet
v	Version
V	Volts
VP1	Viral protein 1
VP1u	Viral protein 1-unique
VP2	Viral protein 2
WRCC	Walter Reed canine cell
$\times g$	Relative centrifugal force



ABSTRAK

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

PENGESANAN BOCAVIRUS DALAM KUCING DAN ANJING MALAYSIA

Oleh

Lee Chee Yien

2016

Penyelia: Prof. Madya Dr Siti Suri Arshad

Penyelia bersama:

Dr Ooi Peck Toung

Dr Gayathri Thevi Selvarajah

Dr Nor Yasmin Abd. Rahaman

Sejak kebelakangan ini, terdapat penemuan novel kumpulan felin bocavirus (FBoV) dan kanin bocavirus (CBoV) yang semakin meningkat di seluruh dunia. Kucing dan anjing mempunyai kaitan yang rapat dalam kehidupan manusia. Namun, tiada penyelidikan berkenaan pengesanan FBoV dan CBoV di Malaysia. Oleh itu, projek ini bermatlamat untuk mengesan bocavirus dalam kucing dan anjing Lembah Klang. Menggunakan teknik persampelan mudah, 4 kucing dan 37 anjing disampel daripada 2 pusat perlindungan haiwan yang berlainan. Sampel tisu merangkumi nod limfa submandibular, paru-paru, buah pinggang, nod limfa mesenteric, usus dan nod limfa inguinal diperolehi daripada nekropsis haiwan diproses dan diuji dengan kaedah PCR konvensional menggunakan primer spesifik menyoar kepada gen *nonstructural 1* (NS1). Berdasarkan analisa PCR, 100% (4/4) kucing dan 24.3% (9/37) anjing masing-masing positif untuk FBoV dan CBoV.

Penjujukan nukleotid separa NS1 telah dilaksanakan untuk 2 produk PCR FBoV dan dibandingkan dengan pencilan rujukan. Analisa filogenetik awal menunjukkan bahawa pencilan FBoV Malaysia adalah berbeza dengan pencilan rujukan. Penyelidikan lanjut berkenaan prevalens dan patologi harus dilakukan untuk memahami sepenuhnya patogenesis FBoV dan CBoV ke atas hos. Kesimpulannya, Malaysia merupakan negara yang kelima dalam pengesanan FBoV dan CBoV.

Kata kunci : felin bocavirus, kanin bocavirus, PCR, penjujukan, 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 BOCAVIRUS IN MALAYSIAN CATS AND DOGS

by

Lee Chee Yien

2016

Supervisor: Assoc. Prof. Dr Siti Suri Arshard**Co-supervisors:****Dr Ooi Peck Toung****Dr Gayathri Thevi Selvarajah****Dr Nor Yasmin Abd. Rahaman**

Recently, there have been an increasing number of novel groups of feline bocavirus (FBoV) and canine bocavirus (CBoV) discoveries around the world. Despite that cats and dogs are highly associated in human living environment, no study has been done to determine the presence of FBoV and CBoV in Malaysia. Thus, this study aimed to detect bocavirus in local cats and dogs in Klang Valley. Using convenience sampling method, 4 cats and 37 dogs were chosen from animal shelters. Tissue samples consisted of submandibular lymph node, lung, kidney, mesenteric lymph node, intestine, and inguinal lymph node obtained from necropsies were processed and subjected to conventional PCR using specific primers targeting the conserved nonstructural protein 1 (NS1) gene. Based on PCR analyses, 100% (4/4) and 24.3% (9/37) of cats and dogs were positive for FBoV and CBoV, respectively. Partial nucleotide sequencing of the NS1 gene were performed on 2 PCR products of FBoV and comparison of sequences were

performed with published isolates. Preliminary phylogenetic analysis revealed that Malaysian FBoV isolates are distinct from reference isolates. Further study on the prevalence and pathology of FBoV and CBoV should be carried out on cats and dogs to fully understand the pathogenesis of FBoV and CBoV in the host. In conclusion, Malaysia is the fifth country to detect FBoV and CBoV.

Key words: feline bocavirus, canine bocavirus, PCR, sequencing, phylogenetic analysis



1.0 INTRODUCTION

Bocavirus is a genus classified within the family of *Parvoviridae*, subfamily of *Parvovirinae*. The name of the genus Bocavirus is a combination of the initials of the two earliest bocavirus discovered; which are the bovine parvovirus (BPV) and canine minute virus (CMV). To date, bocaviruses that have been discovered include canine minute virus (CMV) (Binn *et al.*, 1970), bovine parvovirus (BPV) (Chen *et al.*, 1986), human bocavirus (HBoV) (Allander *et al.*, 2005), 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).

The genus Bocavirus came to significant when a group of Swedish scientist identified human bocavirus (HBoV) in pooled samples from the respiratory tract causing respiratory disease in children (Allander *et al.*, 2005). Over the decade, HBoV have been reported worldwide including Malaysia, when it was first detected in a 13-month-old boy suffering from pneumonia and underlying asthma (Etemadi *et al.*, 2012). Some of the bocaviruses have been associated with diarrheal and respiratory illness in human and other mammals such as dogs, cattle, and pigs (Allander *et al.*, 2005; Kapoor *et al.*, 2012; King *et al.*, 2011) even though pathogenicity is unknown (Martin *et al.*, 2009).

Phylogenetic analyses show that HBoV groups have CMV and BPV in clade, in which it shares a sequence identity of 44.1% and 42.6% respectively (Manteufel & Truyen, 2008). According to Zeng *et al.* (2011), PBoV exhibited sequence identities of 38.0 – 54.7% to HBoV. Since both HBoV and animal bocavirus are

closely related, studies on canine bocavirus or other animal bocaviruses could possibly emulate studies that had been conducted for HBoV; whereby in turn potentially serves as an animal study model for further HBoV research.

Canine minute virus (CMV) or also referred as minute virus of canine (MVC) for certain journals, was the first known bocavirus infecting dogs isolated from fecal sample of a clinically healthy dog (Binn *et al.*, 1970). It has been proven to infect fetuses and cause reproductive disorders. Young animals may suffer severe respiratory infection (Carmichael *et al.*, 1994); while adults may be subclinically infected or show mild diarrhea (Manteufel and Truyen, 2008). Studies on the seroprevalence of CMV suggest that this virus is widespread in many countries (Manteufel and Truyen, 2008).

The second species of dog bocavirus or known as canine bocavirus (CBoV) was identified in 2012 in respiratory samples from diseased and healthy dogs (Kapoor *et al.*, 2012). Three distinctly different strains of CBoV have been identified, provisionally named CBoV (Kapoor *et al.*, 2012), CBoV2 (Bodewes *et al.*, 2014) and CBoV3 (Li *et al.*, 2013). Unlike CMV, pathogenic potential of CBoV remains unknown but it is possibly associated to respiratory disease (Kapoor *et al.*, 2012) and gastrointestinal disease (Bodewes *et al.*, 2014).

Feline bocavirus (FBoV) is a newly discovered bocavirus that infects domestic cats at was first described in Hong Kong in 2012 (Lau *et al.*, 2012). Later, another feline bocavirus denoted as FBoV2 was identified in Portugal (Ng *et al.*, 2014), which is distinctly different from FBoV found in Hong Kong. Since then, no other study that demonstrates the presence of bocavirus in cats have been carried

out and the pathogenicity of feline bocaviruses remains unknown. The clade of feline bocavirus (FBoV and FBoV 2) is most closely related to CMV, CBoV and CsL BoV (Ng *et al.* 2014).

In year 2015, with the positive detection of PBoV in pig population using conventional polymerase chain reaction (PCR) method, this marks the first report of animal bocavirus in Malaysia (Daniel, 2015; Daniel *et al.*, 2015); hence, proving the potential presence of other animal bocaviruses in Malaysia. Despite cats and dogs are the most common domestic pets worldwide that share close habitat with human; no study has been carried out to determine the presence of bocavirus in cats and dogs in Malaysia. The hypotheses for this study is feline and canine bocaviruses are present in Malaysia and could be detected in their tissue samples. Therefore, the main objective of this study is to determine the presence of bocavirus in cats and dogs in Malaysia using conventional PCR method.

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.
- 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 bocavirus and torque teno virus indicate the presence of multiple viral infections in postweaning multisystemic wasting syndrome pigs. *Virus Research*, 152, 59–64.
- Bodewes, R., Lapp, S., Hahn, K., Habierski, A., Förster, C., König, M., Wohlsein, P., Osterhaus, A.D. & Baumgärtner, W. (2014). Novel canine bocavirus strain associated with severe enteritis in a dog litter. *Veterinary microbiology*, 174(1), 1-8.
- 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.
- Choi, J. W., Lee, K. H., Lee, J. I., Lee, M. H., Lee, K. K., & Oem, J. K. (2015). Genetic characteristics of canine bocaviruses in Korean dogs. *Veterinary microbiology*, 179(3), 177-183.
- Daniel, M. J. (2015). *Detection of Porcine Bocavirus in Malaysian Swine Herds* (DVM Thesis). Universiti Putra Malaysia, Serdang, Malaysia.
- Daniel, M. J., Arshad, S. S., Ooi, P. T., & Selvarajah, G. T. (2015). Preliminary Report: Detection of Porcine Bocavirus in Malaysian Swine Herds. *Proceedings of the 7th Malaysia Association of Veterinary Pathology Scientific Conference held on 20-23 August 2015 at Mahkota Hotel, Melaka*, 24.

- 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.
- Garigliany, M., Gilliaux, G., Jolly, S., Casanova, T., Bayrou, C., Gommeren, K., Fett, T., Mauroy, A., Lévy, E., Cassart, D., & Peeters, D. (2016). Feline panleukopenia virus in cerebral neurons of young and adult cats. *BMC veterinary research*, 12(1), 1.
- ICTV Virus Taxonomy. (2014). Retrieved from <http://ictvonline.org/virusTaxonomy.asp>
- Kapoor, A., Mehta, N., Dubovi, E. J., Simmonds, P., Govindasamy, L., Medina, J. L., Street, C., Shields, S., & Lipkin, W. I. (2012). Characterization of novel canine bocaviruses and their association with respiratory disease. *Journal of General Virology*, 93(2), 341-346.
- 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.
- King, A. M., Lefkowitz, E., Adams, M. J., & Carstens, E. B. (2011). *Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. Elsevier, San Diego, CA.
- Lau, S. K., Woo, P. C., Yeung, H. C., Teng, J. 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. *Journal of General Virology*, 93(7), 1573-1582.
- Li, L., Pesavento, P. A., Leutenegger, C. M., Estrada, M., Coffey, L. L., Naccache, S. N., Samayoa, E., Chiu, C., Qiu, J., Wang, C., & Deng, X. (2013). A novel bocavirus in canine liver. *Virology Journal*, 10(1), 1.
- 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.

- Manteufel, J., & Truyen, U. (2008). Animal bocaviruses: a brief review. *Intervirology*, 51(5), 328-334.
- Martin, E. T., Taylor, J., Kuypers, J., Magaret, A., Wald, A., Zerr, D., & Englund, J. A. (2009). Detection of bocavirus in saliva of children with and without respiratory illness. *Journal of clinical microbiology*, 47(12), 4131-4132.
- Mochizuki, M., Hashimoto, M., Hajima, T., Takiguchi, M., Hashimoto, A., Une, Y., Roerink, F., Ohshima, T., Parrish, C. R. and Carmichael, L. E. (2002). Virologic and serologic identification of minute virus of canines (canine parvovirus type 1) from dogs in Japan. *Journal of Clinical Microbiology*, 40(11), 3993-3998.
- Ng, T. F. F., Mesquita, J. R., Nascimento, M. S. J., Kondov, N. O., Wong, W., Reuter, G., Knowles, N. J., Vega, E., Esona, M. D., Deng, X., & Vinje, J. (2014). Feline fecal virome reveals novel and prevalent enteric viruses. *Veterinary microbiology*, 171(1), 102-111.
- Pesavento, P. A., & Murphy, B. G. (2013). Common and emerging infectious diseases in the animal shelter. *Veterinary Pathology Online*, 0300985813511129.
- Pratelli, A., Buonavoglia, D., Tempesta, M., Guarda, F., Carmichael, L., & Buonavoglia, C. (1999). Fatal canine parvovirus type-1 infection in pups from Italy. *Journal of Veterinary Diagnostic Investigation*, 11, 365–367.
- Qiu, X. W., Liu, W. P., Qi, Z. Y., Duan, Z. J., Zheng, L. S., Kuang, Z. Z., Zhang, W. J., & Hou, Y. D. (2008). Phospholipase A2-like activity of human bocavirus VP1 unique region. *Biochemical and Biophysical Research Communications*, 365, 158–163.
- Rinaldo, D., Foti, M., Ginoprelli, T., Orlandella, B. M., & Buonavaglia, D. (2000). Epidemiologia dell' infezione da Parvovirus tipo 1 nel cane: ricerca di anticorpi in cani della Sicilia. *Obiet Docum Vet*, 21, 17–19.
- 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.
- Zádori, Z., Szelei, J., Lacoste, M. C., Li, Y., Gariépy, S., Raymond, P., Allaire, M., Nabi, I.R. and Tijssen, P. (2001). A viral phospholipase A 2 is required for parvovirus infectivity. *Developmental cell*, 1(2), 291-302.

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.

Zhang, W., Li, L., Deng, X., Kapusinszky, B., Pesavento, P. A., & Delwart, E. (2014). Faecal virome of cats in an animal shelter. *Journal of General Virology*, 95(11), 2553-2564.

