



## First Nearly Complete Genome Sequence of Porcine Bocavirus Strain from Malaysia

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**Abstract** | This paper reports the first nearly complete genome sequence of a Malaysian Porcine bocavirus (PBoV) detected in Malaysia from a porcine organ sample. In Malaysia, both Human bocavirus (HBoV) and PBoV have been reported, at prevalence of 5.6% and 90.9% respectively. Most recently, in 2018, PBoV emerged as a zoonosis when a human case of PBoV-related respiratory infection is reported in a child with history of contact with porcine secretion. With this evidence of human infection with PBoV, considering close contact between humans and pigs in the swine industry, further study of PBoV genetic characterization and epidemiology is warranted. This study attempts to obtain the complete genome sequence of a Malaysian PBoV isolate detected in a pig farm in the state of Selangor. A nearly complete sequence was obtained through primer walking and touchdown PCR method. Based on phylogenetic analysis for the nearly complete genome, the Malaysian PBoV strain is genetically characterized as PBoV G3B, sharing a close relationship with a reference PBoV strain from U.S.A. with 98% homology. This finding may facilitate further epidemiological studies and diagnostic development of PBoV in Malaysia.

**Keywords** | Porcine bocavirus, PBoV, Pigs, Malaysia

**Received** | April 11, 2019; **Accepted** | July 18, 2019; **Published** | August 26, 2019

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**Citation** | Lee CY, Tan CY, Tan YJ, Wong JWN, Jacob DM, Lim JX, Ananthan G, Arshad SS, Selvarajah GT, Ooi PT (2019). First nearly complete genome sequence of porcine bocavirus strain from malaysia. *Adv. Anim. Vet. Sci.* 7(9): 776-781.

**DOI** | <http://dx.doi.org/10.17582/journal.aavs/2019/7.9.776.781>

**ISSN (Online)** | 2307-8316; **ISSN (Print)** | 2309-3331

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## INTRODUCTION

The family Parvoviridae is currently classified with two subfamilies: Parvovirinae and Densovirinae, infecting vertebrate and non-vertebrate hosts respectively. Parvovirinae is further classified into five genera: Dependovirus, Bocavirus, Erythrovirus, Parvovirus and Amdovirus (Claude and Fauquet, 2004). Among them, Bocaviruses are considered unique due to the presence of a third open reading frame (ORF) between their non-structural and structural coding regions (Manteufel and Truyen, 2008). Bocaviruses have been detected in human (Allander et al., 2007), swine (Liu et al., 2014; Amimo et al., 2016), canine (Decaro et al., 2012), feline (Lau et al., 2012), bovine

(Abinanti and Warfield, 1961), rat (Babkin et al., 2013) and wildlife including gorillas (Lau et al., 2017), California sea lions (Li et al., 2011) as well as bats (Lau et al., 2016). Since the discovery of human bocavirus (HBoV) in 2005 (Allander et al., 2005), it has been associated with human respiratory tract gastrointestinal infections (Schildgen et al., 2008; Kapoor, et al., 2009; Schildgen, 2010; Kantola et al., 2011; Khamrin et al., 2012). In Malaysia, HBoV was first detected in 2012, in a 13-month-old boy with pneumonia and underlying asthma (Etemadi et al., 2012). Subsequent study reported a HBoV prevalence of 5.6% among 125 cases of acute respiratory distress (Tg Rogayah et al., 2014). Most recently, in 2018, PBoV had emerged as a zoonosis; causing respiratory infection in a child with history

**Table 1:** Primers used in PCR for the PBoV target

Primer	Orientation	Sequence	Bases	Tm (°C)	Position based on KF025386 (Approx.)
a	Forward	GTGCGCCGAGGACTTTGA	18	59.4	1951 -> 1968
b	Reverse	CGCAAAGTTACCGCCAC	18	58.5	3245 <- 3262
c	Forward	TATCGACTCTTTGCAGTCTGAC	22	56.9	3217 -> 3238
d	Reverse	TGACGTAGATGGTTCCCGGA	20	59.3	4801 <- 4820
e	Forward	TTGGGCTGTCTGATCAGGA	19	56.9	234 -> 252
f	Reverse	CTGAGATAGTGAGTGATCGGCT	22	58.1	1018 <- 1039
g	Forward	ACAGGCAGCCGATCACTCACTAT	23	62.6	1008 -> 1030
h	Reverse	CTCGTTCCCTCCCATCAGACACTT	23	60.9	1728 <- 1706
i	Forward	GGAGATAATGATAGACACGCCTT	23	56.9	3 -> 25
j	Forward	GATTTTGTGCTCTACGCTCAAGGAC	25	61.4	2060 -> 2084
k	Reverse	CCSRGAAGAYGTTCCATGCTC	22	62.3	2467 <- 2488
m	Reverse	ATAAAGTCTTGATCACCTT	19	47.3	3201 <- 3219

of contact with porcine secretion (Safamanesh et al., 2018). With this evidence of human infection with PBoV, considering close contact between humans and pigs in the swine industry, further study of PBoV genetic characterization and epidemiology is warranted.

Porcine bocavirus was first identified in lymph nodes of pigs in Sweden, co-detected with Porcine circovirus 2 (PCV2) (Blomström et al., 2009). Detection was reported in wide range of samples including saliva, sera, nasopharyngeal swab, feces, lymph nodes, tonsil, lung, kidney, spleen and liver (Choi et al., 2014; Zhou et al., 2014; Gunn et al., 2015), suggestive of systemic infections. It was linked to gastrointestinal and respiratory signs as well as encephalomyelitis (Pfankuche et al., 2016). The pathogenic pathways of PBoV are rather obscured, with multiple reports of co-infections (Blomström et al., 2009; Shan et al., 2011; Ndze et al., 2013). It was suggested to be indirectly related to porcine post-weaning multisystemic wasting syndrome (PMWS) and other swine diseases as a triggering factor (Blomström et al., 2009). In Malaysia, PBoV was first detected in 2017 in clinically compromised pigs (Jacob et al., 2018). As our continual effort in genetic characterization of Malaysian PBoV isolate, this study attempts to obtain the complete genome sequence by primer walking. In summary, here we described the nearly complete genome of one PBoV field strain isolated from Selangor, Malaysia; and its phylogenetic relationship compared to reference bocavirus strains.

## MATERIALS AND METHODS

### SAMPLE, DNA EXTRACTION AND PCR ASSAY

From the tissue samples that were detected positive of PBoV previously (Jacob et al., 2018), total DNA was isolated using Patho Gene-spin DNA/RNA Extraction Kit (iNtRON Biotechnology, South Korea). A series of spe-

cific PCR primers were designed (synthesized by Bioneer, South Korea) to obtain the initial target sequence to be used as a template to ‘walk’ the length of the target (Table 1). The primers were paired in various combinations resulting in fragments of varying sizes (Table 2). Real-time PCR was performed using NexQ-9300 NEXpro™ qPCR 2x MasterMix Solution (EvaGreen, U.S.A.) with touchdown cycling conditions to increase annealing specificity and yield of the target fragments (Table 3).

**Table 2:** Combination of PCR primers and their expected PCR product size

Primer Combination	Nucleotide Positions	Expected Size (bp)
i+f	4->1039	1036
e+h	234->1728	1495
g+b	1008->3262	2255
j+m	2060->3219	1160
c+d	3217->4820	1604
a+k	1951->2488	538

**Table 3:** Touchdown qPCR cycling conditions

Phase	Step	Temperature	Duration	Cycle (s)
Denaturation	Melting	95°C	300 secs	1
Touchdown	Melting	95°C	15 secs	16
	Annealing	67°C - 0.5°C/cycle	55 secs	
	Extension	73°C	140 secs	
Normal	Melting	95°C	15 secs	19
	Annealing	61°C	55 secs	
	Extension	73°C	140 secs	
Extension	Final Extension	72°C	600 secs	1

SEQUENCING AND PHYLOGENETIC ANALYSIS

Due to multiple signals being generated from direct sequencing of the fragments, TA cloning using pLUG-Prime TA-Cloning Kit (iNtRON Biotechnology, South Korea) was employed to obtain clean sequences and ascertain whether multiple variants of the PBoV target were present. The near complete genome of PBoV was deduced from the best overlaps spanning the entire sequence of the target PBoV. Phylogenetic analysis of the nucleotide sequences of complete or near complete genome of PBoV and other bocaviruses available in NCBI GenBank database was conducted using MEGA7.0.26 software (Kumar, Stecher and Tamura, 2018) using Maximum Likelihood method based on Tamura-Nei model with 1000 bootstrap replicates (Saitou and Nei, 1987).

RESULTS

A previous selected positive PBoV sample (Jacob et al., 2018) was subjected to real-time PCR and a nearly complete genome sequence comprising 4836 nucleotide with G+C content of 52% was obtained. Sequence fragments generated from PCR of the primer combinations (Table 2) are shown in Figure 1. Nucleotide BLAST analysis of the initial sequencing data indicated that the target had a high homology of 98% with PBoV Group 3 lineage. The near complete genome of Malaysian PBoV had been submitted to GenBank under accession number MK467456. The nucleotide sequence of the near complete genome is shown in Figure 2.

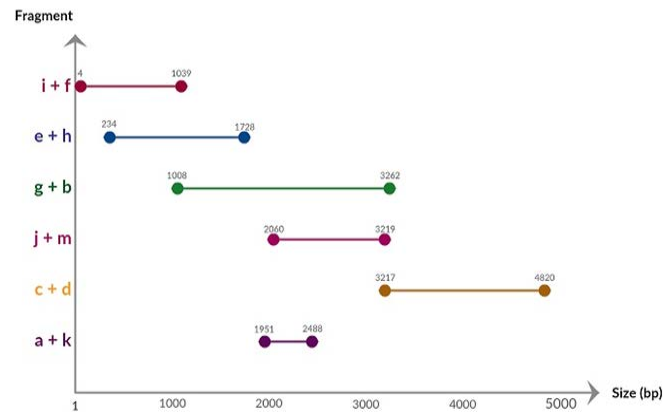


Figure 1: Chart showing the sequence fragments generated from PCR of primer combinations and their respective nucleotide position in the PBoV genome.

The result of phylogenetic analysis comparing Malaysian PBoV isolate with complete or near complete genome for PBoV and other bocaviruses is shown in Figure 3. The Malaysian PBoV isolate MK467456 (indicated by round bullet) formed a clade with PBoV strains KF025382 and KF025386 (indicated by triangle bullets), both isolated from domestic pigs in U.S.A. The result indicates a strong

relationship between Malaysian PBoV with these two aboard strains.

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TTCCGGGCACGCAACTACGGGATGAGGTTGATATGACGTTGATGGTTCTGATGACAGAGGGGCTATATAAGGAAGAAACAGGAAGAAGAGCTCATTG
CGGGGGACATCGSAGTGTTCACATCGCGGGAACGAGACGCTTGGGGACATCCCTTCCAGTGGCAAGACAGCTCCGCGAAGTACTGCTGTATATATC
TGCTGGCATGGCTTCTGCTGGAGTGGGCTGTCTGATCAGGAATGATTAATTTTGGCATGGCAATACAGGCTTAAAGGCTCCATCTATACATATGTGATC
CCGCTGGATATAAGGACTGGAGAGTTCATGAGAGAGCTCTTATGGACATTTTATCCAAAGATTTATCTACATCTGTGATGACATGTAGAAAGAG
CAGATCATCAACGATGTGCAATATGATTTGAAAGCCGATTTAGCATGAGGCTCAGAAATCAGCGGCTGGTCAAGTCTCTGTGAGCTGCTGACATGGCC
GTCAACGGCTGTTTCAACTGAAGCAGCAATCAAAATCCGCTCTATAGCTGCTTCCAGCAGTCCGAGATTGGGAGGAGACCATCGACATCATATTTGTGTGT
GGGGAGATGGCTGACTGATTTAACGCCAAAAGAGCAGCTTTACATTTGCCAAGTATTTTGGAAACGATGCTGATCTGGAACACATCAGGAGGCTGT
CAGCATCCGACCAAGTGTCAATATGCCATCTGGTACAGCTGGCTGTCTGTGGAGAGGCTGAGGCTCATCAGGATACGAGCTGACGCTGCTGACCTATAG
AGATAGACCAACGAGTGTCAAGCTCAACGCTGATGCTACAGCTACATCAACCAATCTGCTGCCAAGAAATAGGATGCTGACCACTGCTGGGCGCAG
ATATCTGCACACCGAGGCTGCTATTTTGACCAAGAGAGCTCATGTGCACTGACAGTTCAGAGGAGGCTGATGCTGACAGAAAGGCTGCTGACCACTG
ACCGCTGCTCATGACAGCAGCGAGACACCGGAAACCGGCTGCAATAGATGCGCGCTGGGCTGATCTTCTCAGTGGGTGAGACCTGCTAGCGAGAG
GGAACTTACAGAGAGAGCATCAAAAGATCACCCGAAACAATGTGTGATGATAGATACGCTACAGATGAGCAGGATCAAGTTTATGTGTCAAAAGAGGAGCTGA
CGATGCTTCAACGAGTGTGATGATTTTGGAGAGCTACCGGGGCGCTTCCAGAGCTGAGGAGGCTTGGACATGCATCGAGTGGGCTCACTCGCTCC
TACTCGGCTCTCGGGTCACTTCAACAGCTTTCCAGAGAAACAATTTTCAAGCCGGAAGAAAGGATAGGCTTCTGAATCCAGGCTCAACATCTACAT
AGCTCGGCACTGGGTGGCAGTGTGGTGGAGAGAGGCGGGAACAGAACACCATCTGTTTCTATGGTCCGGCCAGTACGGGAAAAACCAATCTGGCTAAG
GGCTCAGGAGTGGGGAAGATCTATGGTTGTGTCAATCACCTCAACAGGAGTTTGTCTTAAAGATTCAGAGAAAGCTCATATCTGTTGGGAGGAG
CGGTGATGACACAGGATGTAGACACCGGCTAAGTGTCTGATGGAGGAGACACTTCAGATGATGACAGAAACACAGAGAGGAGGAGGAGGAGGAGGAGG
CTTCTGCTTACGAGCACTATGACATTTACACAGTACGAGGTTGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
TTCTAAGAGGCGCTGCTCAAAATTTGGAGAGATTTACAGGCTGATGTGGCTGATGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
CAGAAGAAATGGAGCTGACCGTGTGCCAACGCTTTCTTAACTGTCTACTGTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT
CGCTGTGGTGGTACTGCTGCTACTACTACACAGGAGGAGGATGGAGTGGAGTGGCTGATCAGGATGAGGCTTCCGCTGCTGCTGCTGCTGCTGCTGCTGCT
GTGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
GAGCAACTCTGAGTGAATCATGACTGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
TCCCTACTCGAGGATCCAGGCGAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
CCGAGCGCGGAGCTCTGCTCAGCTGACCAAGTGTCTCGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
AGCAGCAGAGTGGTAAGAAACAGATCCGGCTCAGGTTTCCGCGGAAACCGAAGAGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
AAGAATTTGAGAAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
CATTTCTTATAAGCGTGTCTGAGCAGATGTACCAGCTATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT
GCATATAGCGGCTGACTGCTGCTGAACTATGCTCTTCTGCTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT
CGCGGTCAGTTCGCGGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
AAACACGATCAAGCTATAATATATCAATAAAGGTTAAATCCCTAACTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT
TTTGGCGGTAATTTGCAAAAGGCTGCTTATCAAAAGGCAATACGCCCGGCTCAACGAGCAGAAAGACCGGGGAGGAGGAGGAGGAGGAGGAGGAGG
AGCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
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ACGGCAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
CTTCTGACTGGGCTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT
CAACAGAGAAACAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
AAGGAGCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
TCCGTACAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
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CATGATGCGCAACAGTGTGGGAGTCCGCGAGATCTCCAGATACCAATCCCATCTGGCAAGATACCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
GAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
    
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Figure 2: The near complete genome of Malaysian PBoV isolate.

DISCUSSION

Grouping system of PBoV varies due to the genetic diversities in PBoV isolates. International Committee on Taxonomy of Viruses (ICTV) classified Porcine bocaviruses into four species namely *Ungulate bocaparvovirus 1* to 4 (Cotmore et al., 2013). The current ICTV criterion for classification of bocaviruses defines separate species as a group of individuals with less than 95% homologous non-structural gene DNA sequence (Cotmore et al., 2014). Based on that, the NS1 genes of PBoVs would divide them into ten species; but their NP1 genes only divide them into nine (Zhai et al., 2015). Recently, a VP1-based classification has been proposed (Yang et al., 2012), dividing PBoVs into three groups: group 1 or PBoV1, G1 (represented by PBo-likeV), group 2 or PBoV2, G2 (PBoV1/2-CHN and PBoV2-A6), and group 3 or PBoV3, G3 (PBoV3/4-UK, PBoV3/4-HK, and PBoV3C).

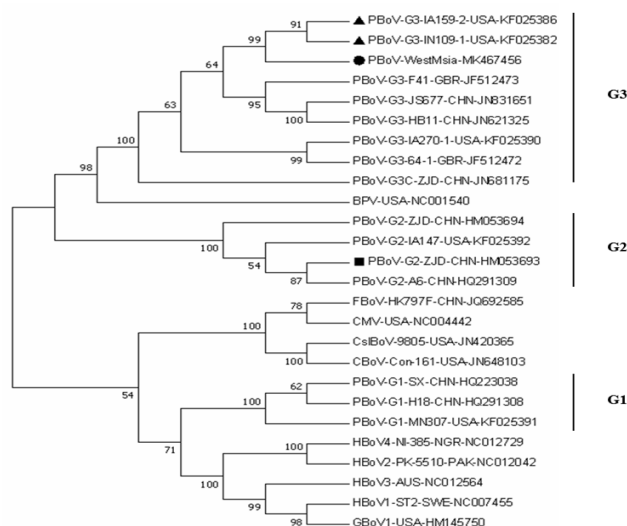
From the Figure 3, the Malaysian PBoV isolate MK467456 (indicated by round bullet) showed a strong relationship with the both United States PBoV strains KF025382 and KF025386 (indicated by triangle bullets). This relationship could be linked to the importation source of Malaysian

breeding herd, given that majority of the breeder pigs used in Malaysian swine farms are imported from U.S.A (Singh and Fong, 2014). Considering the possibility of introduction of this PBoV G3 isolate through commercial trading by importation of semen and breeder stock, this virus could be expected to be circulating in Malaysian commercial swine herd, as shown by a pilot molecular detection of PBoV in Malaysia reporting a very high prevalence of 90.9% (Jacob et al., 2018). Therefore, understanding the genetic characteristics of the Malaysian isolate of PBoV could contribute to the management of bocavirus infection in the local scenario. Screening of breeder animals and semen for bocavirus prior importation and strict biosecurity measures upon arrival may help to prevent any further introduction of new bocavirus strains into the country. In terms of NS1 gene sequence, these 3 strains share 98% of their nucleotide identity. Thus, this Malaysian PBoV isolate can be classified as same species with reference strains KF025382 and KF025386 as Porcine bocavirus G3B.

the HBoV, as compared to G1 which shares a large clade with HBoV. Although the Malaysian PBoV is only distinctly related to HBoV, possible zoonotic risk still cannot be ruled out. Furthermore, strain HM053693 (indicated by square bullet) reported to be closest to the strain in the first human case of PBoV (Safamanesh et al., 2018), is classified as PBoV G2 that shares a clade with G3. This might suggest potential zoonotic risk. Thus, this genetic characterization of Malaysian PBoV G3B isolate could be useful for the start of epidemiology studies of PBoV in Malaysia: further studies to determine whether PBoV G1 and G3 is present in Malaysia, as well as whether the Malaysian PBoV strain poses zoonotic risk.

## CONCLUSION

Publication of near complete genome sequences allows researchers to trace the presence of new, important emerging viruses and determine their genetic distribution to plan relevant control programs. With the characterization of Malaysian Porcine bocavirus isolate as strain PBoV G3B having close relationship with U.S.A. reference strains KF025382 and KF025386, we suggest that routine screening of imported breeders could be helpful to monitor the status of new emerging disease in Malaysian swine farms. Further studies are required to establish the clinical impact of the current Malaysian PBoV G3B strain as well as to determine presence of other PBoV strains in Malaysia. In conclusion, PBoV is present in Malaysia and the Malaysian isolate is confirmed to be of PBoV G3B strain.



**Figure 3:** Phylogenetic tree of Malaysian PBoV isolate MK467456 with 25 nucleotide sequences of complete or near complete genome of PBoV and other bocaviruses. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The vertical lines indicate PBoV grouping based on VP-1 classification. Round bullet designates Malaysian PBoV sequence generated from the present study; triangle bullets designate the two U.S.A. PBoV strains closest to Malaysian strain; square bullet designates the China PBoV strain closest to the PBoV reported in human infection (Safamamesh et al., 2018). Virus names are as follows: PBoV, *Porcine bocavirus*; BPV, *Bovine parvovirus*; HBoV, *Human bocavirus*; GBoV, *Gorilla bocavirus*; CMV, *Canine minute virus*; FBoV, *Feline bocavirus*; CBoV, *Canine bocavirus*; CslBoV, *California sea lion bocavirus*.

## STATEMENT OF ETHICAL STANDARDS

This research project was approved by the Institutional Animal Care and Use Committee (IACUC), with reference number UPM/IACUC/AUP-R063/2014.

## ACKNOWLEDGEMENT

The authors are thankful to the Government of Malaysia and its Ministry of Higher Education for funding this research through the Fundamental Research Grants Scheme (FRGS) (Grant no. FRGS 2014-1-5524541) and Universiti Putra Malaysia through the Putra Grant – Putra Graduate Initiative (Grant no. GP-IPS/2016/9492500).

## CONFLICT OF INTEREST

The authors declared that no conflict of interest in this research study.

Bocaviruses from G2 and G3 are more distantly related to

LCY, TCY, TYJ contributed in manuscript writing and data interpretation. LJX, DM and JWWN conducted the research work. OPT, SS, GTS and DM contributed to methodology design and manuscript editing.

## REFERENCES

- Abinanti FR, Warfield MS (1961). Recovery of a hemadsorbing virus (HADEN) from the gastrointestinal tract of calves. *Virology*. 14: 288. [https://doi.org/10.1016/0042-6822\(61\)90206-9](https://doi.org/10.1016/0042-6822(61)90206-9)
- Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B (2005). Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc. Natl. Acad. Sci.* 102(36): 12891-12896. <https://doi.org/10.1073/pnas.0504666102>
- Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Usterback R, van den Hoogen BG (2007). Human bocavirus and acute wheezing in children. *Clin. Infect. Dis.* 44(7): 904-910. <https://doi.org/10.1086/512196>
- Amimo JO, El Zowalaty ME, Githae D, Wamalwa M, Djikeng A, Nasrallah GK (2016). Metagenomic analysis demonstrates the diversity of the fecal virome in asymptomatic pigs in East Africa. *Arch. Virol.* 161(4): 887-897. <https://doi.org/10.1007/s00705-016-2819-6>
- Babkin IV, Tyumentsev AI, Tikunov AY, Kurilshikov AM, Ryabchikova EI, Zhirakovskaya EV, Tikunova NV (2013). Evolutionary time-scale of primate bocaviruses. *Infect. Genet. Evol.* 14: 265-274. <https://doi.org/10.1016/j.meegid.2012.12.023>
- Blomström AL, 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 Res.* 146(1-2): 125-129. <https://doi.org/10.1016/j.virusres.2009.09.006>
- Choi MG, Park SJ, Nguyen VG, Chung HC, Kim AR, Park BK (2014). Molecular detection and genetic analysis of porcine bocavirus in Korean domestic swine herds. *Arch. Virol.* 159(6): 1487-1492. <https://doi.org/10.1007/s00705-013-1944-8>
- Claude M, Fauquet M (2004). Virus taxonomy: 8th report of the ICTV. Elsevier Sci. Technol.
- Cotmore SF, Agbandje-McKenna M, Chiorini JA, Gatherer D, Mukha DV, Pintel DJ, Tijssen P (2013). Rationalization and extension of the taxonomy of the family Parvoviridae. ICTV official taxonomy: Updates since the 8th Report, code.
- Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, Qiu J, Davison AJ (2014). The family parvoviridae. *Arch. Virol.* 159(5): 1239-1247. <https://doi.org/10.1007/s00705-013-1914-1>
- Decaro N, Amorisco F, Lenoci D, Lovero A, Colaianni ML, Losurdo M, Buonavoglia C (2012). Molecular characterization of Canineminute virus associated with neonatal mortality in a litter of Jack Russell terrier dogs. *J. Vet. Diagn. Invest.* 24(4): 755-758. <https://doi.org/10.1177/1040638712445776>
- Etemadi MR, Azizi Jalilian, F, 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. *Med. J. Malaysia.* 67(4): 433-434.

- Gunn L, Collins PJ, Fanning S, McKillen J, Morgan J, Staines A, O'Shea H (2015). Detection and characterisation of novel bocavirus (genus Bocaparvovirus) and gastroenteritis viruses from asymptomatic pigs in Ireland. *Infect. Ecol. Epidemiol.* 5(1): 27270. <https://doi.org/10.3402/iee.v5.27270>
- Jacob DM, Lee CY, Arshad SS, Selvarajah GT, Bande F, Ong BL, Ooi PT (2018). First molecular detection of porcine bocavirus in Malaysia. *Trop. Anim. Health Prod.* 50(4): 733-739. <https://doi.org/10.1007/s11250-017-1489-z>
- Kantola K, Hedman L, Arthur J, Alibeto A, Delwart E, Jartti T, Söderlund-Venermo M (2011). Seroepidemiology of human bocaviruses 1-4. *J. Infect. Dis.* 204(9): 1403-1412. <https://doi.org/10.1093/infdis/jir525>
- Kapoor A, Simmonds P, Slikas E, Li L, Bodhidatta L, Sethabutr O, Bukbuk DN (2010). Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *J. Infect. Dis.* 201(11): 1633-1643. <https://doi.org/10.1086/652416>
- Kapoor A, Slikas E, Simmonds P, Chieochansin T, Naem A, Shaikat S, Delwart E (2009). A newly identified bocavirus species in human stool. *J. Infect. Dis.* 199(2): 196-200. <https://doi.org/10.1086/595831>
- Khamrin P, Malasao R, Chaimongkol N, Ukarapol N, Kongsricharoen T, Okitsu S, Maneekarn N (2012). Circulating of human bocavirus 1, 2, 3, and 4 in pediatric patients with acute gastroenteritis in Thailand. *Infect. Genet. Evol.* 12(3): 565-569. <https://doi.org/10.1016/j.meegid.2012.01.025>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35(6): 1547-1549.
- Lau SK, Woo PC, Yeung HC, Teng JL, Wu Y, Bai R, Yuen KY (2012). Identification and characterization of bocaviruses in cats and dogs reveals a novel feline bocavirus and a novel genetic group of canine bocavirus. *J. Gen. Virol.* 93(7): 1573-1582. <https://doi.org/10.1099/vir.0.042531-0>
- Lau SK, Ahmed SS, Yeung HC, Li KS, Fan RY, Cheng TY, Woo PC (2016). Identification and interspecies transmission of a novel bocaparvovirus among different bat species in China. *J. Gen. Virol.* 97(12): 3345-3358. <https://doi.org/10.1099/jgv.0.000645>
- Lau SK, Yeung HC, Li KS, Lam CS, Cai JP, Yuen MC, Yuen KY (2017). Identification and genomic characterization of a novel rat bocavirus from brown rats in China. *Infect. Genet. Evol.* 47: 68-76. <https://doi.org/10.1016/j.meegid.2016.11.014>
- Li L, Shan T, Wang C, Côté C, Kolman J, Onions D, Delwart E (2011). The fecal viral flora of California sea lions. *J. Virol.* 85(19): 9909-9917. <https://doi.org/10.1128/JVI.05026-11>
- 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. *Sci. World J.* 2014. <https://doi.org/10.1155/2014/947084>
- Manteufel J, Truyen U (2008). Animal bocaviruses: a brief review. *Intervirology.* 51(5): 328-334. <https://doi.org/10.1159/000173734>
- Ndze VN, Cadar D, Cságola A, Kisfali P, Kovács E, Farkas S, Bányai K (2013). Detection of novel porcine bocaviruses in fecal samples of asymptomatic pigs in Cameroon. *Infect. Genet. Evol.* 17: 277-282. <https://doi.org/10.1016/j.meegid.2012.12.023>

- Pfankuche VM, Bodewes R, Hahn K, Puff C, Beineke A, Habierski A, Baumgärtner W (2016). Porcine bocavirus infection associated with encephalomyelitis in a pig, Germany. *Emerg. Infect. Dis.* 22(7): 1310. <https://doi.org/10.3201/eid2207.152049>
- Safamanesh S, Azimian A, Shakeri A, Ghazvini K, Jamehdar SA, Khosrojerdi M, Youssefi M (2018). Detection of porcine bocavirus from a child with acute respiratory tract infection. *Pediatr. Infect. Dis. J.* 37(12): e338-e339. <https://doi.org/10.1097/INF.0000000000002003>
- Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mole. Biol. Evol.* 4(4): 406-425.
- Schildgen O, Müller A, Allander T, Mackay IM, Völz S, Kupfer B, Simon A (2008). Human bocavirus: passenger or pathogen in acute respiratory tract infections?. *Clin. Microbiol. Rev.* 21(2): 291-304. <https://doi.org/10.1128/CMR.00030-07>
- Schildgen O (2010). Erratum: Human bocavirus: Increasing evidence for virulence. *Pediatr. Pulmo.* 45(3): 312-313. <https://doi.org/10.1002/ppul.21218>
- Shan T, Lan D, Li L, Wang C, Cui L, Zhang W, Delwart E (2011). Genomic characterization and high prevalence of bocaviruses in swine. *PLoS One.* 6(4): e17292. <https://doi.org/10.1371/journal.pone.0017292>
- Singh MSG, Fong RWJ (2014). Swine breeding and production in Malaysia. In *Proc. of Int. Symp. on Recent Progress in Swine Breeding and Raising Technologies* (pp. 3-4).
- Tg Rogayah TAR, Fauziah MK, Apandi YM, Zarina MZ, Nur Iznawati AR, Nur Azrenawaty MN, Zainah S (2014). Molecular epidemiology of human bocavirus in children with acute respiratory infections in Malaysia. *Innov. J. Med. Health Sci.* 4: 319-323.
- Yang WZ, Yu JM, Li JS, Cheng WX, Huang CP, Duan ZJ (2012). Genome characterization of a novel porcine bocavirus. *Arch. Virol.* 157(11): 2125-2132. <https://doi.org/10.1007/s00705-012-1407-7>
- Zhai SL, Li XP, Chen QL, Chen SN, Wei WK, Luo ML (2015). Sequence Comparison and Phylogenetic Analysis of Several Emerging Porcine Bocaviruses and a Proposal Regarding Nomenclature. *Virol. Mycol.* 4(1).
- Zhou F, Sun H, Wang Y (2014). Porcine bocavirus: achievements in the past five years. *J. Viruses.* 6(12): 4946-4960. <https://doi.org/10.3390/v6124946>