



**MOLECULAR DETECTION, RISK FACTOR AND PUBLIC AWARENESS  
OF AVIAN BORN VIRUS FROM CAPTIVE AND NON-CAPTIVE BIRDS  
IN PENINSULAR MALAYSIA**

By

**SYAMSIAH BINTI MOHD LUTPI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Degree of Master  
of Veterinary Science**

**November 2021**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

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**November 2021**

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**Faculty : Veterinary Medicine**

Proventricular dilatation disease (PDD) is a fatal disorder and one of the threatening diseases among captive birds and wild birds. This disease is caused by avian bornavirus (ABV) which belongs to the family of *Bornaviridae*. ABV has been identified in psittacine and non-psittacine birds as well as waterfowls. Birds with PDD may show signs of gastrointestinal tract deficit (e.g., weight loss, regurgitation, passage of undigested food in faeces), or neurological dysfunction (e.g., ataxia, abnormal gait, proprioceptive defects) or even both. ABV is also present in healthy birds with no clinical signs of PDD. ABV infections have been reported in several countries such as Japan, Brazil, Thailand, South Africa, Germany, Canada, Denmark and North-eastern USA among the group of captives as well as free ranging birds of clinically diseased or healthy birds. Among five species of ABV, Parrot bornavirus (PaBV) 2 and 4 in the group species of *Psittaciform 1 bornavirus* are the most notably causing the disease. In Malaysia, there was no study of ABV reported in captive as well as wild birds. Therefore, three main objectives of this study were: 1) To determine the molecular prevalence of avian bornavirus in captive and wild birds in Peninsular Malaysia by nucleic acid detection through reverse transcription polymerase chain reaction (RT-PCR) assay by targeting the M gene; 2) To determine the risk factors associated with ABV infection among captive and non-captive birds that was molecularly detected positive and 3) To assess the level of public awareness on ABV and its associated diseases via a set of knowledge, attitude, practice (KAP) questionnaires..

Total of 344 cloacal swabs or faeces were collected from various species of captive and non-captive birds at various regions of Peninsular Malaysia, and were subjected to detection of ABV using RT-PCR assay. The positive amplicons were subjected for sequencing for ABV genotypes analyses.

Meanwhile, online KAP questionnaires were distributed by using google form platform to the pet bird's owners.

Molecular prevalence studies revealed that 4.5% (9/201) of pet birds were ABV positive with two birds were presenting PDD-like signs and the other seven birds were healthy and had no outward clinical signs of PDD, whereas 0% (0/143) in waterfowls. From the 9 positive pet birds, all were identified to be PaBV-2, which is closest to ABV isolates EU781967 originated from USA. Among the risk factors analyzed, several risk factors which include the category, family, species, age, place as well as management are found to show association with the ABV positivity among the birds.

Besides that, a total of 87% (158/181) of respondents were interviewed regarding the diseases (ABV, PDD) using the KAP questionnaires. The majority of respondents have low knowledge on ABV and PDD with a percentage of 32.9% answered correctly. However, they showed a positive attitude and good practice with a total percentage of 60.8% and 94.9% respectively. Meanwhile, the association between knowledge, attitude and practice showed that there was a significant association between knowledge-attitude and also attitude-practice ( $p < 0.05$ ).

In conclusion, this study proved that avian bornavirus (ABV) causes proventricular dilatation disease (PDD) among a group of pet birds is present in Peninsular Malaysia but at low prevalence rate. Furthermore, in addition to the useful databases obtained from this study, the level of public awareness on the importance of avian bornavirus that causes fatal disorder among a wide range of bird species is satisfactorily achieved.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

**PENGESANAN MOLEKUL, FAKTOR RISIKO DAN KESEDARAN  
AWAM TERHADAP VIRUS BORNAVIRUS DARIPADA BURUNG  
KURUNGAN DAN BURUNG BEBAS DI SEMENANJUNG MALAYSIA**

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Penyakit Dilatasi Proventikulus (PDD) adalah penyakit yang menyebabkan kematian dan ancaman utama dalam kalangan burung kurungan dan burung liar. Penyakit ini disebabkan oleh virus 'Avian Bornavirus' (ABV) yang tergolong dalam keluarga '*Bornaviridae*'. Penyakit ini telah dikesan pada burung dalam kumpulan psittacine dan bukan psittacine termasuk unggas air. Burung dengan PDD akan menunjukkan tanda-tanda gangguan pada saluran pencernaan (seperti: penurunan berat badan, regurgitasi, pengeluaran makanan yang tidak dicerna melalui najis) atau sistem neurologi yang tidak berfungsi dengan baik (seperti: tidak seimbang, gaya berjalan yang tidak normal, kecacatan proprioceptif) atau kedua-duanya sekali. Selain itu, ABV juga dijumpai pada burung yang sihat tanpa menunjukkan tanda-tanda klinikal PDD. Jangkitan ABV telah dilaporkan dari pelbagai negara seperti Jepun, Brazil, Thailand, Afrika Selatan, Jerman, Kanada, Denmark dan AS timur laut dalam kalangan burung peliharaan termasuk burung liar sama ada burung ini menunjukkan gejala klinikal mahupun sihat tanpa gejala. Di antara lima spesies ABV, Parrot bornavirus (PaBV) 2 dan 4 dalam kumpulan spesies *Psittaciform 1 Bornavirus* adalah penyebab utama penyakit ini. Di Malaysia, tidak ada lagi kajian mengenai ABV yang dilaporkan pada burung peliharaan dan juga burung liar. Oleh itu, tiga objektif utama untuk kajian ini adalah: 1) Untuk mengenalpasti kewujudan virus ABV dalam kalangan burung peliharaan dan burung tidak ditawan melalui kaedah pengesanan molekul RNA iaitu kaedah tindak balas rantai polimerase transkripsi terbalik dengan mensasarkan gen M; 2) Untuk menentukan faktor risiko yang berkaitan dengan jangkitan ABV di antara burung kurungan dan burung bebas yang secara molekul dikesan positif dan 3) Untuk menilai tahap kesedaran masyarakat terhadap ABV dan

penyakit yang berkaitan dengannya melalui satu set soal selidik pengetahuan, sikap, amalan (KAP).

Sebanyak 344 calitan pada kloaka atau najis dikumpulkan dari pelbagai spesies burung tawanan dan bukan tawanan di pelbagai negeri di Semenanjung Malaysia untuk pengesanan molekul ABV melalui RT-PCR. Hasil positif sampel dari keputusan RT-PCR seterusnya dihantar untuk mengenal pasti genotip ABV tertentu. Sementara itu, soal selidik KAP dilakukan dengan menggunakan platform borang google dan disebarluaskan dalam kalangan pemilik burung.

Hasil kajian pengesanan molekul menunjukkan bahawa 4.5% (9/201) burung peliharaan positif ABV di mana dua burung menunjukkan tanda klinikal PDD dan tujuh burung lagi sihat tanpa tanda klinikal, manakala 0% (0/143) pada unggas air. Sebanyak 9 sampel positif yang diperoleh dikenal pasti berkait dengan PaBV-2, yang mana paling hampir dengan isolat ABV EU781967 berasal dari negara USA. Di antara faktor risiko yang dianalisis, beberapa faktor risiko yang merangkumi kategori, keluarga, spesies, umur, tempat dan pengurusan didapati menunjukkan kaitan dengan positif ABV di antara burung.

Selain itu, sejumlah 87% (158/181) responden ditemu ramah mengenai penyakit (ABV, PDD) melalui soal selidik KAP. Hasil yang diperoleh menunjukkan majoriti responden mempunyai pengetahuan yang rendah mengenai ABV dan PDD dengan peratusan 32.9% menjawabnya dengan betul. Namun, mereka menunjukkan sikap positif dan amalan baik dengan jumlah peratusan masing-masing 60.8% dan 94.9%. Sementara itu, perkaitan antara pengetahuan, sikap dan amalan menunjukkan bahawa terdapat hubungan yang signifikan antara pengetahuan-sikap dan juga sikap-amalan ( $p < 0.05$ ).

Sebagai kesimpulan, kajian ini membuktikan bahawa virus Bornavirus Avian (ABV) yang menyebabkan Penyakit Dilatasi Proventikulus (PDD) dalam sekumpulan burung peliharaan terdapat di Semenanjung Malaysia tetapi pada kadar prevalensinya rendah. Di samping itu, sebagai tambahan maklumat data berguna yang diperoleh dari kajian ini, tahap kesedaran masyarakat mengenai pentingnya virus ABV yang menyebabkan kematian dalam pelbagai jenis burung juga dapat dicapai.

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## LIST OF ABBREVIATIONS

ABV	Avian bornavirus
ABV1	Avian bornavirus genotype 1
ABV2	Avian bornavirus genotype 2
ABV3	Avian bornavirus genotype 3
ABV4	Avian bornavirus genotype 4
ABV5	Avian bornavirus genotype 5
ABV6	Avian bornavirus genotype 6
ABV7	Avian bornavirus genotype 7
ABBV-1	Aquatic bird bornavirus1
ABBV-2	Aquatic bird bornavirus2
ABV-CG	Canada goose genotype
ABV-MALL	Mallard genotype
BLAST	Basic Local Alignment Search Tool
BDV	Bornavirus disease virus
BoDV-1	Borna disease virus 1
cDNA	Complementary deoxyribonucleic acid
COX-2	Cyclooxygenase-2
CnBv-1	Canary bornavirus 1
CnBv-2	Canary bornavirus 2
CnBv-3	Canary bornavirus 3
EsBV-1	Estrildid finch bornavirus
IHC	Immunohistochemistry
KAP	Knowledge, Attitude and Practice



NCBI	National Centre for Biotechnology Information
PaBV-1	Parrot bornavirus 1
PaBV-2	Parrot bornavirus 2
PaBV-3	Parrot bornavirus 3
PaBV-4	Parrot bornavirus 4
PaBV-5	Parrot bornavirus 5
PaBV-7	Parrot bornavirus 7
PDD	Proventricular dilatation disease
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SPSS	Statistical Package for Social Sciences

# CHAPTER 1

## INTRODUCTION

### 1.1 Overview

Nowadays, the interest to pet birds were slowly increase as it is a small creature with long lives as companions which require small living spaces, relatively inexpensive to feed and some of the birds have good socialization (Kalhagen, 2019). According to Douglas Main (2021) in a National Geographic article, new research approximates around 50 billion to 430 billion birds living on Earth with an estimation of almost 10,000 species of birds (Cox, 2021). In Asia, the number of bird species recorded was 3784 with 837 species found in Malaysia (Lepage, 2021). The most popular companion pet birds are parakeet/budgie, cockatiel, finch, lovebird, monk parakeet and African grey parrot because they require only a small space as they are small in size as well as highly intelligent (Kalhagen, 2020). Several diseases of pet birds have been reported to be either non-infectious (e.g., nutritional, metabolic, toxic) or infectious disease due to bacteria, fungal or virus infections. One of the infectious diseases caused by virus infection is the Proventricular Dilatation Disease (PDD) (Reavill & Dorrestein, 2018).

In the late 1970s, PDD was a new disease in psittacine birds where it was first detected in macaws imported from Bolivia to the United States (Redmann *et al.*, 2011; Nedorost *et al.*, 2012; Donatti *et al.*, 2014; Hoppes *et al.*, 2013). Initially, PDD was named as Wasting Macaw Syndrome as this disease only occurred in macaws. Later, PDD was suggested and used to describe the syndrome because it was also discovered in most psittacine groups other than macaws (Gregory *et al.*, 1994). During that period, the aetiologic agent remains unknown until in 2008, the Avian bornavirus (ABV) was detected in psittacine birds infected with this disease (Thomsen *et al.*, 2015; Guo *et al.*, 2014; Hoppes *et al.*, 2013). It has been confirmed by two independent research groups where they reported on the finding of a novel Bornavirus from the tissues of a parrot that was infected and died from PDD (Guo *et al.*, 2015; Gancz *et al.*, 2010). A year later (2009) in Ontario, Canada, ABV which was originally detected in captive Psittaciformes was first identified in waterfowls, specifically free ranging Canada Geese (*Branta canadensis*) and Trumpeter Swans (*Cygnus buccinator*), which showed similar clinical signs of PDD (Delnatte *et al.*, 2014; Thomsen *et al.*, 2015).

Avian bornavirus (ABV) is an enveloped, non-segmented negative-sense RNA virus, in the order of *Mononegavirales* and under the family of *Bornaviridae* (Guo *et al.*, 2014). Avian bornavirus (ABV) was reclassified into five specific species which are *Passeriform 1 bornavirus*, *Passeriform 2*

*bornavirus*, *Psittaciform 1 bornavirus* (consists of parrot bornaviruses PaBV 1, 2, 3, 4 and 7), *Psittaciform 2 bornavirus* as well as *Waterbird 1 bornavirus* (Sa-ardta *et al.*, 2019; Guo & Tizard, 2015). Among the parrot bornaviruses in the group of *Psittaciform 1 bornavirus*, PaBV-2 and PaBV-4 were found to be causing disease the most (Guo & Tizard, 2015).

This disease has been reported in more than 80 species of birds (Nedorost *et al.*, 2012; Donatti *et al.*, 2014), mainly under members of the Psittacidae family such as macaws (*Ara sp*), African gray parrots (*Psittacus erithacus*), Amazon parrots (*Amazona sp*), conures (*Aratinga sp*) and also family of Cacatuidae such as cockatoos (*Cacatua sp*) and cockatiels (*Nymphicus hollandicus*) (Gregory *et al.*, 1994; Gancz *et al.*, 2010). Among these species of birds, the most common species affected with ABV were the blue and gold macaws (*Ara ararauna*) and African grey parrots (*Psittacus erithacus*) (Gregory *et al.*, 1994), conures, *Poicephalus* spp., and cockatoos (Reavill & Dorrestein, 2018). Apart from that, PDD has not only been detected in captive birds, but also in waterfowls, such as swans, geese, gulls and ducks (Guo *et al.*, 2014).

Clinically, birds with PDD affected by this virus show clinical signs related to malfunction of the digestive system or neurologic system or a combination of both systems (Rubbenstroth *et al.*, 2014; Gancz *et al.*, 2010). Birds with gastrointestinal tract dysfunction may show signs of weight loss, lethargy, reduced appetite, constant or intermittent regurgitation, delayed crop emptying, passage of undigested food and proventricular dilatation (Gray *et al.*, 2010; Gregory *et al.*, 1994; Donatti *et al.*, 2014). Meanwhile, ABV infection in the central nervous system may show signs of ataxia, abnormal head movements, proprioceptive deficits, seizures, and blindness (Gray *et al.*, 2010; Gregory *et al.*, 1994, Mirhosseini *et al.*, 2011). However, in many cases, birds infected with ABV may not show any clinically related symptoms and many become healthy carriers and shed the virus in their droppings for years and the likelihood of PDD causing latent infection is strongly supported in previous researches. (Guo *et al.*, 2014; Monaco *et al.*, 2012, Raghav *et al.*, 2010).

## **1.2 Problem statement**

Proventricular dilatation disease (PDD) caused by the avian bornavirus (ABV) is a fatal disorder that causes lesions at the gastrointestinal organ and central nervous system in a wide range of psittacine birds, including wild birds. Avian bornavirus (ABV) may be found in clinically affected birds and also healthy birds with no clinical signs of PDD. Increasing interest in captive birds as well as movement of wild birds from one place to another may increase possibility of infection by this virus. Currently, there was no study conducted on prevalence and other related work of ABV in Malaysia. Thus, the possibility of healthy captive or wild birds being affected by this virus is still unknown.

### 1.3 Hypothesis

- Ho: Avian bornavirus in captive birds and non-captive birds in Peninsular Malaysia is detected through molecular detection.
- Ha: Avian bornavirus in captive birds and non-captive birds in Peninsular Malaysia is not detected through molecular detection.
- Ho: There is association between risk factors and ABV molecular positivity.
- Ha: There is no association between risk factors and ABV molecular positivity.
- Ho: The levels of knowledge, attitude and practice towards avian bornavirus among the study population is low.
- Ha: The levels of knowledge, attitude and practice towards avian bornavirus among the study population is high.

### 1.4 Objectives

1. To detect the presence of avian bornavirus (ABV) among captive and non-captive birds in Peninsular Malaysia using the RT-PCR test.
2. To determine risk factors associated with ABV infection among captive and non-captive birds in Peninsular Malaysia.
3. To assess public awareness and disease related knowledge on ABV via KAP questionnaire.

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