UNIVERSITI PUTRA MALAYSIA

DETECTION OF CANINE DISTEMPER VIRUS (CDV) IN WILD ARBOREAL SMALL MAMMALS IN FOREST SURROUNDING FRUIT ORCHARDS OF BUKIT BESI, TERENGGANU

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DETECTION OF CANINE DISTEMPER VIRUS (CDV) IN WILD ARBOREAL SMALL MAMMALS IN FOREST SURROUNDING FRUIT ORCHARDS OF BUKIT BESI, TERENGGANU

HEW ZIE KAI

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Detection of Canine Distemper Virus (CDV) in Wild Arboreal Small Mammals in Forest Surrounding Fruit Orchards of Bukit Besi, Terengganu” by Hew Zie Kai and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement of the course VPD 4999-Project.

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DEDICATION

This thesis is dedicated to my supervisor, Dr. Tengku Rinalfi Putra Tengku Azizan, my co-supervisor, Dr. Khor Khuan Hua, Dr Bryan Andrew Lazarus, Muhammad Farris Mohd Sadali, seniors, my family, and friends.

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CONTENTS

LIST OF ABBREVIATIONS

BSAVA	British Small Animal Veterinary Association
CDV	Canine Distemper Virus
CNS	Central Nervous System
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
FYP	Final Year Project
F	Fusion
H	Hemagglutinin
IACUC	Institutional Animal Care and Use Committee
L	Large protein
M	Matrix protein
N	Nucleocapsid
P	Phosphoprotein
RBC	Red blood cell
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SNT	Serum neutralization test

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek Tahun Akhir.

PENGESANAN CANINE DISTEMPER VIRUS (CDV) PADA HAIWAN MAMALIA KECIL ARBOREAL LIAR DI HUTAN SEKELILINGI DUSUN BUAH BUKIT BESI, TERENGGANU

Oleh

Hew Zie Kai

2023

Penyelia: Dr. Tengku Rinalfi Putra Tengku Azizan

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Canine Distemper Virus (CDV) pernah dianggap sebagai patogen yang hanya menyerang anjing, tetapi kini diketahui sebagai patogen berbilang perumah yang tersebar secara meluas yang menghasilkan kematian yang ketara dalam pelbagai spesies karnivor, terutamanya harimau Malaya yang terancam. Penumpahan virus CDV sangat menular dan terutamanya merebak melalui laluan oronasal melalui aerosol. Selain itu, CDV boleh menjangkiti pelbagai perumah, menjadikannya patogen baru yang ketara. Oleh itu, kajian ini dijalankan untuk menentukan data

prevalens untuk CDV dalam mamalia kecil arboreal liar seperti tupai dan musang, yang telah dispekulasi dikaitkan dengan peningkatan pendedahan CDV kepada harimau Malaya. Hutan sekitar kebun buah-buahan di Bukit Besi, Terengganu, dipilih kerana disyaki terdapat jangkitan CDV pada harimau Malaya pada 2019. Lima belas haiwan ditangkap dengan perangkap sangkar diletakkan di kawasan habitat. Sapuan konjungtiva dan hidung diperolehi apabila mamalia kecil yang terperangkap telah dibius. Sampel yang diperolehi disimpan pada suhu 4°C, diangkut, dan diekstrak untuk analisis selanjutnya. Semua sampel kemudiannya diuji untuk antigen menggunakan tindak balas rantai polimerase transkripsi terbalik (RT-PCR) dan elektroforesis gel. Akibatnya, semua sampel telah diuji negatif; oleh itu, mamalia kecil ini bebas daripada CDV. Oleh kerana kekurangan kajian yang berkaitan di Malaysia, penemuan ini telah memberikan jurang pengetahuan untuk data prevalens CDV dalam harimau Malaya di negara kita. Kesimpulannya, keputusan yang diperoleh dalam kajian ini adalah tidak mencukupi untuk mewakili prevalens CDV di seluruh penduduk Bukit Besi, Terengganu. Oleh itu, ketepatan pengesanan CDV boleh ditingkatkan dengan peningkatan saiz sampel.

Kata kunci: CDV; mamalia kecil arboreal liar; RT-PCR; Wild arboreal small mammals; Malaysia

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 - Final Year Project.

DETECTION OF CANINE DISTEMPER VIRUS (CDV) IN WILD ARBOREAL SMALL MAMMALS IN FOREST SURROUNDING FRUIT ORCHARDS OF BUKIT BESI, TERENGGANU

By

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2023

Supervisor: Dr. Tengku Rinalfi Putra Tengku Azizan

Co-Supervisor: Dr. Khor Kuan Hua

Canine distemper virus (CDV) was once thought to be a pathogen that only affects dogs, but it is now known to be a widely dispersed multi-host pathogen that produces significant mortality in various carnivore species, especially the endangered Malayan tigers. Viral shedding of CDV is highly contagious and primarily spreads through the oronasal pathway via aerosol. Besides, CDV can infects various hosts, making it a significant emerging pathogen. Thus, the present study was conducted to determine prevalence data for CDV in wild arboreal small mammals such as squirrels and civets, which was speculated associated with

increased exposure of CDV to Malayan tigers. Forests surrounding fruit orchards in Bukit Besi, Terengganu, were selected as there was a suspected CDV infection in Malayan tigers in 2019. Fifteen animals were captured with cage traps placed in the habitat area. Conjunctival and nasal swabs were obtained as the trapped small mammals were anesthetised. Samples obtained were stored at 4°C, transported, and extracted for further analysis. All the samples were then tested for antigen using reverse transcription polymerase chain reaction (RT-PCR) and gel electrophoresis. As a result, all the samples were tested negative; hence, these small mammals were free of CDV. Due to a lack of relevant studies in Malaysia, these findings have provided a knowledge gap for CDV prevalence data in Malayan tigers in our country. In conclusion, the results obtained in this study are insufficient to represent the prevalence of CDV in the entire population of Bukit Besi, Terengganu. Therefore, the accuracy of CDV detection could be increased with an increase in sample size.

Keywords: CDV; Malayan tigers; RT-PCR; Wild arboreal small mammals; Malaysia

1.0 INTRODUCTION

Canine distemper virus (CDV) is a negative sense, single-stranded RNA virus in the family Paramyxoviridae. It is classified within the genus Morbillivirus, which contains other highly pathogenic viruses, such as the measles virus, which affects humans and other primates. (Martella et al., 2008). CDV was once thought to be a pathogen that only affects dogs, but it is now known to be a widely dispersed multi-host pathogen that produces significant mortality in various carnivore species. (Loots et al., 2017)

Wild carnivores have been repeatedly linked to CDV (Martinez-Gutierrez et al., 2016). Social animals like lions (Panthera leo) (Roelke-Parker et al., 1996), grey wolves (Canis lupus) (Di Sabatino et al., 2014), and African wild dogs (Lycoan pictus) (Goller et al., 2010) have been known to experience the most dramatic outbreaks (Munson et al., 2008). Within this environment, illness can spread quickly and occasionally lead to the extinction of nearby populations (Timm et al.,2009) (Van de Bildt et al.,2002) (Gordon ,2015). Nevertheless, losing the wild black-footed ferret (Mustela nigripes) suggests that solitary carnivores face similar dangers (Thorne et al.,1988). Random effects can affect any small, remote population, and the cumulative death from CDV can have a significant influence (Robinson et al., 2015). The presence of CDV was found to increase the 50-year extinction probability of small populations of Amur tigers (P. tigris altaica) by 65%, when distemper was first recognised as a threat to wild tigers (Gilbert et al., 2014). After that, the virus was found in free-roaming tigers and leopards living in Indonesia and India (Mulia et al.,2021) (Rahman et al., 2022) (Kadam et al., 2022). In wild felines, characteristic symptoms have been linked to a neurological disease that progresses and usually ends in seizures and death (Rahman et al.,2022) (Sulikhan et al.,2018) (Seimon et al., 2013). Between 2019 and 2020, two Malayan tigers (P. tigris

jacksoni) showed up to Malaysian wildlife authorities and passed away with signs of CDV. Unfortunately, there is currently little research on transmission of CDV in Malayan tigers.

In terms of characteristics, CDV is similar to other RNA viruses. It has a low replication fidelity mutation, which is spread by a wide range of carnivore species and other arboreal animals, such as rodents and primates (Martinez-Gutierrez et al., 2016). Usually, it is transmitted through direct contact or contact with infectious material (Sykes et al.,2014). In some species, morbidity and mortality rates can reach as high as 95% in populations that have not been previously exposed (Uhl et al.,2019). Animals thatrecover will have strong immunity towards CDV. In addition, the virus needs to be maintained with a large population (Viana et al.,2015), and threatened animals are usually due to secondary spill-over from other more common hosts (Almberg et al.,2010) like dogs (Butler et al., 2014) and arboreal animals like squirrels and civets. This is because their population is high, which has a higher chance of transmission to other animals. The complexity of CDV reservoirs varies, with some populations having a higher diversity of hosts than others. (Haydon et al., 2002) In some cases, dogs only play a small role in a broad community of hosts; sometimes, they may have little or no role (Oleaga Á et al., 2022; Viana et al., 2015)

Canine distemper can be investigated using various techniques, with the most suitable one depending on the specific situation and available samples. One effective method is RT-PCR, which can detect the virus during its acute phase when it is present in faeces and other bodily fluids. (Meli, 2010) Another helpful technique is sequencing, which can provide valuable information on genetic lineages and transmission pathways. (Mulia et al., 2021; Seimon et al., 2013) Typically, shedding

is a temporary occurrence, and it may have already ceased by the time an animal displays neurological symptoms. In order to identify viruses in post-mortem samples, RT-PCR or immunohistochemistry can be utilised, particularly in the brain and lymphoid tissues (Kadam et al., 2022). As free-ranging animal populations are seldom handled, and active infections may go undetected, serological assays are particularly useful in identifying previous infections (Mulia et al., 2021). Antibodies are generally detectable 10–20 days after infection (Zhao et al., ……..) and typically persist for life (Greene et al., 2006). Nowadays, commercial test kits like ELISA are specifically designed for certain species with specific antibody markers like anti-dog IgG or IgM (Bergmann M. et al., 2021) (Waner T. et al.,2003) and the results on other species are not verified yet. The only accurate serological test available for felids is the serum neutralisation test (SNT).

1.1 OBJECTIVES

To detect Canine Distemper Virus (CDV) in Wild Arboreal Small Mammals which is associated with exposure to Malayan Tigers

1.2 HYPOTHESIS

The null hypothesis (H_o) for this study was:

H_o = Canine distemper virus (CDV) is not detected in Wild Arboreal Small Mammals in Forest Surrounding Fruit Orchards of Bukit Besi, Terengganu

The alternative hypothesis (H_a) for this study was:

H_a= Canine distemper virus (CDV) is detected in Wild Arboreal Small Mammals in Forest Surrounding Fruit Orchards of Bukit Besi, Terengganu

2.0 LITERATURE REVIEW

2.1 CANINE DISTEMPER VIRUS (CDV)

Canine distemper virus (CDV) is the causative agent of canine distemper. It belongs to the Paramyxoviridae family and the genus Morbillivirus (Murphy et al., 2012). This disease has been identified in dogs since 1760 and is highly contagious, causing acute fever (MacLachlan et al., 2011). CDV has a wide range of cell tropisms, including epithelial, lymphoid, and neurological cells, which can lead to systemic infections and affect various organs such as the central nervous system (CNS), gastrointestinal tract, urinary tract, skin, and lymphatic system (Lempp et al., 2014). The order Carnivora, which includes families such as Canidae (dog, dingo, fox, coyote, jackal, wolf), Procyonidae (raccoon, coatimundi), Mustelidae (weasel, ferret, fishers, mink, skunk, badger, marten, otter), Ursidae (giant panda), Ailuridae (red panda), and a variety of members of the family Felidae (lions, leopards, cheetahs, tigers), comprises the majority of CDV's host range (MacLachlan et al., 2011; Martinez-Gutierrez et al., 2016). Given the wide range of species impacted by CDV, there has been significant research on cross-species transmission between wildlife and domesticated animals. This research focuses on understanding the interactions between these species and establishing their phylogenetic relationships. (Beineke et al., 2015) The genome structure of CDV consists of six transcription units (N-P-M-F-H-L) arranged in a linear form (Kolakofsky et al., 2016) Every protein has a specific role in the viral cycle and replication. According to the expression of the CDV gene, the nucleocapsid (N) protein encapsidates the genomic RNA, and N acts as a template for transcription, viral polymerase (L) and its cofactor, the phosphoprotein (P) is for the replication. The N, L and P proteins form the ribonucleoprotein (RNP) complex with viral RNA (von Messling et al., 2001).

Meanwhile, the CDV envelope consists of two integral membrane proteins, the hemagglutinin (H), and fusion (F) proteins, and a membrane-associated protein M (da Fontoura et al., 2016). The H protein has emerged as the primary focus for studying the variability and evolution of CDV. Among CDV strains, it is regarded as the gene with the highest genetic variation, exhibiting up to 11% divergence at the nucleotide level. This characteristic has made it possible to conduct CDV phylogenetic studies based on genetic divergence and molecular epidemiology focused on understanding the evolutionary dynamics. (von Messling et al. , 2001)

Clinical Signs

The clinical signs of CDV include catarrhal and nervous signs. In the acute stage, viruses are present in all the animals' secretions (Avila et al., 2015). After that, the development of a cutaneous rash, severe nasal and ocular discharge, anorexia, and conjunctivitis can also be seen. Gastrointestinal and respiratory signs also follow, frequently exacerbated by secondary bacterial infections and neurological disorders (MacLachlan et al.,2011). The neurological symptoms of CDV infection in animals may include tetraparesis or plegia, myoclonus, ataxia, nystagmus, and postural reaction deficits (Amude et al., 2007)

However, an improved immune system can promote animal recovery by increasing the production of virus-specific neutralising antibodies (Vandevelde et al., 2005). Despite eliminating the virus from various organs and peripheral blood, CDV can persist in specific tissues such as the uvea, CNS, lymphoid organs, and footpads. Additionally, some infected animals may experience delayed and impaired development, as well as a moderate immune response with subtle early clinical signs (Schobesberger M. et al., 2005). Certain disturbances may be observed.

Typically, dogs cannot survive CNS pathologies, but in rare cases, they may recover and experience lifelong neurological symptoms (Lempp et al., 2014).

Source and Transmission

CDV can be transmitted to Malayan tigers through domestic dogs as they serve as a reservoir of CDV (Govindan et al., 2020; Truong et al., 2022). The overlapping roaming areas of domestic dogs (Butler et al., 2014) and wildlife may lead to a higher exposure. The virus transmits from dogs to other domestic animals, such as cats and rodents, and then to wild animals like wild rodents and carnivores, including squirrels, civets and leopards, which are prey animals of wild tigers (Beineke et al., 2015).

CDV is a multi-cellular pathogen that can infect three different types of host cells: lymphoid, epithelial, and neurological. It can be transmitted through inhalation of aerosol droplets or airborne virus particles and direct contact with bodily fluids or contact with contaminated fomites (de Vries et al., 2017). like other enveloped viruses, CDV is quickly inactivated in the environment, and transmission primarily occurs through direct contact with animals or exposure to infectious aerosols. Usually, secretions and excretions, such as urine, faeces, saliva, and respiratory secretions, have high titer value of the virus, which can detect the virus quickly (G. Elia et al., 2006)

Diagnosis

CDV can be diagnosed in ante-mortem or post-mortem. In antemortem diagnosis, conjunctival, nasal, blood and urine samples are collected. The samples can then be tested using either RT-PCR or ELISA. According to Frisk et al. in 1999, RT-PCR is a highly specific and sensitive method for early and safe antemortem

diagnosis of distemper, regardless of the form of distemper, clinical signs, pathological findings, neutralising antibody titer and viral antigen distribution. In contrast, a serological test is not as accurate as a molecular test as it does not only detect current infections but also past infections. For post-mortem diagnosis, the brain, urinary bladder, skin and lymphoid tissues like the spleen can be taken and tested with RT-PCR or Immunohistochemistry Test (Kadam et al., 2022). Besides, nested RT-PCR is even better as it is equipped with specific probes which can scharacterise the lineages of CDV and differentiate the field and vaccine strains of CDV (Martella et al., 2007).

Prevention

Vaccination in domesticated dogs is currently the most effective way to control CDV transmission (Rikula U et al.,2007). This prevents domestic dogs from transmitting CDV to free-roaming wildlife when in contact. CDV vaccines contain modified-live virus (MLV), killed or recombinant canarypox vector vaccine with targeted CDV genes. In the United States, vaccination is not administered to free-roaming wildlife unless federal and state authorities decided that such vaccination may benefit these endangered species before releasing back into the wild. In the 1960s through the 1980s, zoo animals and endangered species were immunised against CDV with killed vaccines (KV) (Montali R.J. et al.,1983). After vaccination with the KV, the development of virus-neutralising titers was low, and the outbreaks of CDV infection have killed several exotic species that had received the vaccination. MLV has rarely been used in the United States to control disease in endangered species and display animals in zoologic parks because they are often fatal to many wildlife and zoo animals (Cleaveland et al., 2006). Later, many North American zoological institutions started to use the rCDV vaccine to vaccinate those at-risk

species with the univalent canarypox vectored recombinant distemper vaccine, Purevax Ferret (Merial Inc) when it was licensed and marketed successfully in 2001 (Bronson et al., 2008). Currently, when CDV is endemic in local wildlife, the American Association of Zoo Veterinarians' Distemper Vaccine subcommittee will encourage the extra label use of the rCDV PureVax Ferret Distemper Vaccine (Merial, Inc) in all susceptible zoological display animals.

2.2 POPULATION STATUS OF MALAYAN TIGERS

The Malayan tiger (Panthera tigris jacksoni), which is extremely endangered, is projected to go extinct in the wild within the next ten years due to an intermediate population collapse. The number of individuals is estimated to be less than 200 in Malaysia's fragmented and isolated rainforest habitats. Being a large carnivore, the Malayan tiger is crucial to the stability of rainforest ecosystems because it balances the interactions of plants, herbivores, and predators. As the embodiment of strength and royal authority in Malaysia, the species is prominently featured on the nation's coat of arms (DWNP, 2008).

The Malayan tiger population is currently confronted with significant and conspicuous extinction risks due to human-caused disturbances such as poaching, expansion of industrial agriculture, commercial logging, and human settlement. Additionally, environmental disruptions, including disturbances, decline in habitat quality, and pollution, pose a threat. Furthermore, the trade of illegal tiger products in traditional Chinese medicine (Kawanishi, K., 2015; Clements R. et al., 2010) and diseases (DWNP, 2019; Yung et al., 2021) further contribute to this critical situation.

Nevertheless, Malaysia's government has consistently prioritised Malayan tiger conservation through the Department of Wildlife and National Parks (DWNP). 2017 the Malaysian Mammal Red List was released, indicating that the DWNP had classified the Malayan tiger as a critically endangered and fully protected species (DWNP, 2017)

3.0 MATERIAL AND METHODS

3.1 ANIMAL ETHICS

This study was conducted under the supervision of the institution's veterinarian and in accordance with the guidelines outlined in UPM's Code of Practice for the Care and Use of Animals for Scientific Purposes. They complied with the current guidelines for animal care and use, which were approved by the Institutional Animal Care and Use Committee (IACUC) under AUP number: UPM/IACUC/AUP-U041/2023 dated 31st July 2023. The captured animals were sedated with drugs in accordance with guidelines from the 10th edition of BSAVA Small Animal Formulary and then released back to nature after it is ensured to be healthy and fit enough to be released. None of the animals were harm or killed in this study.

3.2 PERMIT

This study was conducted with approval from the Wildlife Department Malaysia (PERHILITAN) under permit number: JPHL&TN(IP): 100-34/1.24 Jld2011**.** Rules and regulation from PERHILITAN was abided under the supervision of the institution's veterinarian.

3.3 LOCATION

The location chosen was reserved forest surrounding fruit orchards of Kampung Besul, Bukit Besi in Terengganu (Coordinate: 4.70692,103.1770). Bukit Besi is located 33km from Dungun city and 77km from Kuala Terengganu. The area is mostly flat with an average elevation of 23m. Geologically, Bukit Besi has argillaceous and arenaceous layers. It consists of slate, shale, and argillite. (Sarman M. et al., 2019). This location was chosen due to previous case of CDV detection in

Awang Besul, our friendly Malayan tiger. Besides, it has a good biodiversity with varieties of small wild arboreal animals like plantain squirrels, tree squirrels and palm civets.

3.4 SAMPLE COLLECTION

Cage trap was set up with bait like fruits or nuts and place in roaming area of small wild arboreal animals. Once the animals were captured, the body weight of the animals was estimated through observation. Then, drugs dosage was calculated before anaesthetising the animals. In this study, xylazine and ketamine were used as chemical restraint. In the meantime, the temperament and physical appearance of the animals were noted down. After the animals are anaesthetised, the length of the animals was measured with a measuring tape and recorded. Then, conjunctival and nasal samples were taken with sterile swabs and kept in viral transport media immediately. The samples then stored in an ice box to maintain at 4°C and transported back to UPM Virology Lab for further analysis.

3.5 SAMPLE PROCESSING

Firstly, the RNA from the virus was isolated with NucleoSpin® RNA virus kit. This is because CDV is a RNA virus, so it has to be extracted prior to the test. Then, SensiFAST cDNA Synthesis Kit is used to synthesize cDNA from viral RNA using Reverse Transcription Polymerase Chain Reaction (RT-PCR). Later, DNA was amplified with thermal cycler for 1½ hours after adding MyTaq™ Red Mix DNA polymerase. MyTaq™ was used as it has an increased affinity for DNA, enabling reliable amplification from even very low amounts of template. As compared to other PCR templates, it can deliver very high yield of PCR amplification. After the samples are processed, DNA ladder and RedSafe™ nucleic acid staining solution are added

to the agarose gel solution to act as indicator of the results. The samples then pipetted into well of agarose gel model once the agarose gel model is formed. Lastly, gel electrophoresis was run to separate the DNA fragments. The gel was then exposed to UV light to visualize the DNA bands and the results were taken with a gel documentation system. In gel electrophoresis, loading dyes have three main functions. Firstly, they provide density for the sample to sink into the gel. Secondly, the dyes add colour to the sample and make loading easier. Finally, the dyes flow through the gel at consistent rates, making it possible to estimate the migration distance of the DNA fragments. (Lee et al.,2012)

4.0 RESULTS

Throughout the sampling, a total of 15 wild arboreal small mammals were captured from the reserved forest surrounding fruit orchards of Kampung Besul, Bukit Besi in Terengganu. The most captured animals were tree squirrels (n=9), followed by 3 palm civets and 3 plantain squirrels (Figure 1).

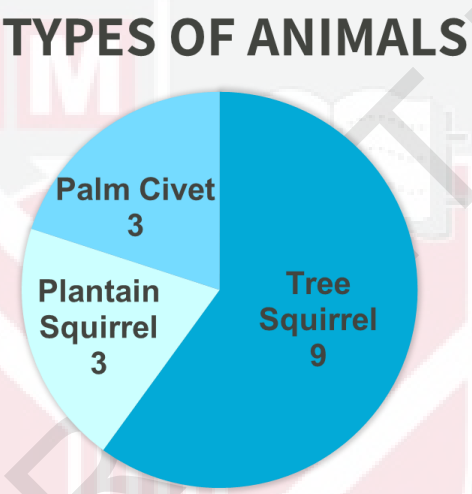

Figure 1: Pie chart of types of animals captured.

Then, all samples were undergone reverse transcription polymerase chain reaction (RT-PCR) test to detect the antigen of CDV. Based on the results from gel electrophoresis, all samples were tested negative from CDV (Figure 2). The results were justified with a valid DNA ladder, positive control and negative control. The results were then tabulated in a table. (Table 1)

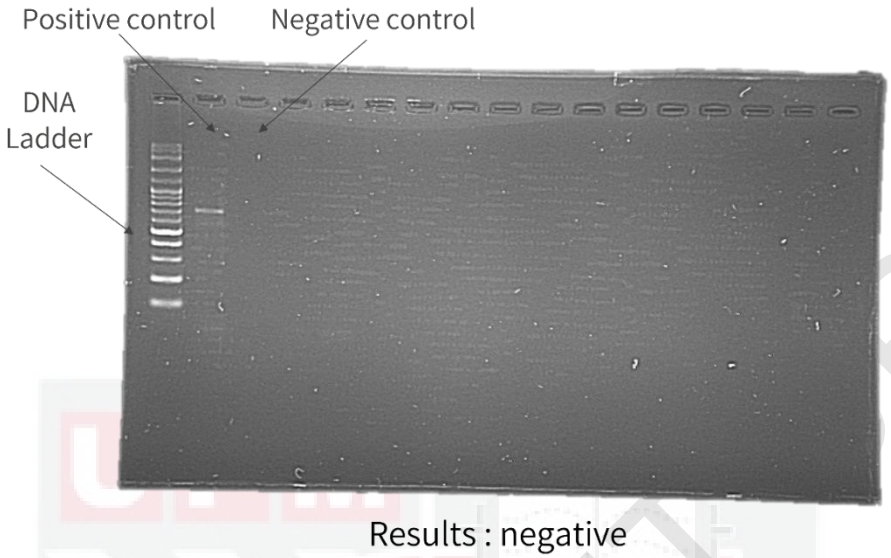

Figure 2: Image of gel electrophoresis result with DNA ladder, positive control & negative control.

Results	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TS9
Positive (+)									
Negative (-)	✓	✓	✓	✓	✓	✓	✓	✓	✓

Results	PS1	PS2	PS3	PC1	PC2	PC3
Positive (+)						
Negative (-)	✓	✓	✓	✓	✓	✓

Table 1: Results of CDV detection in reserved forest surrounding fruit orchards of Kampung Besul, Bukit Besi in Terengganu. TS: Tree Squirrel, PS: Plantain Squirrel, PC: Palm Civet.

5.0 DISCUSSION

In the past 80 years, Malaysians have witnessed the extinction of several species of animals including the Sumatran rhinoceros (Dicerorhinus sumatrensis) which was last seen in 2020, the Banteng (Bos javanicus) and the Java rhinoceros (Rhinoceros sondaicus) in the 1950s and 1932, respectively. (Dennis et al., 2021). Currently, the Malayan tiger population is also encountering the threat of extinction. Hence, this study aims to give insights into the potential connection between the detection of CDV in wild arboreal small mammals and the decline of the Malayan tiger population.

In 2019, the Malayan tiger population was threatened by an outbreak of canine distemper, a highly infectious disease. According to Sim (2020), this disease has already claimed the lives of two wild Malayan tigers in Malaysia. The first incident occurred on July 19, 2019, when a male tiger in Kampung Besul, Terengganu, succumbed to the disease despite receiving treatment from the Department of Wildlife and National Parks (DWNP). Sadly, the tiger passed away on July 23, 2019. The second incident took place on May 1, 2020, when another Malayan tiger was found dead at Ladang Aramijaya, Mersing Johor. While the cause of death has not been officially confirmed, it is suspected to be canine distemper. This disease is believed to be closely linked to other species of animals, although its exact origin cannot be determined.

Canine distemper virus (CDV) is a single-stranded RNA virus that can be detected using RT-PCR. Samples such as conjunctival, nasal, and rectal swabs, urine, and post-mortem tissues (including lymph nodes, tonsils, brain, lungs, spleen, kidneys, and skin) can be used for detection. (Elia G. et al., 2015) It is important to collect a complete set of samples as this increases the likelihood of detecting the

virus and helps to rule out other causes and identify co-infections. (Munsen L*. et al*., 2008)

In a recent article, infections in different types of felids, such as tigers and leopards were found by Indian researchers using RT-PCR in Uttar Pradesh (UP), which shares a southern border with Nepal (Kadam R.G. et al., 2022). After partial hemagglutinin (H) genes were amplified and sequenced, two tiger isolates from UP were discovered to be closely linked to an isolate of dogs from the same area, sharing 99 percent identity. All three isolates belonged to the genetic lineage known as Asia 3. Interestingly, they were not grouped with an isolate of palm civet from UP; instead, they showed a stronger relationship with strains originating from Germany (Kadam R.G. et al., 2022). This suggests that there are several strains of the disease spreading among wildlife around the world, including Malaysia, and that more than one species of animal is being affected. In the future, isolates from Malaysia could be used for research on transboundary transmission and as a comparison to this discovery.

CDV can easily deteriorate in the environment, making direct contact the main way it is transmitted. This can occur in social animals through fighting, grooming, and sharing food. Solitary carnivores, on the other hand, have fewer opportunities for intra-species transmission due to their limited contact with other members of their species. In these cases, transmission from other animals becomes more significant. The possibility of interspecies transmission through predation has been raised, as it provides the intimate contact required for the virus to spread (Gilbert M. et al., 2020). Domestic dogs are known carriers of CDV (Govindan, V. P. et al., 2020; Truong et al., 2022), and it is possible that the overlapping roaming areas of domestic dogs and wildlife could lead to a higher level of exposure. CDV

can rapidly mutate and shed, making it possible for the virus to transmit from dogs to other domestic animals, such as cats and rodents, and then to wild animals like wild rodents and carnivores, including squirrels, civets, and leopards, which are prey animals of wild tigers as well. (Beineke A et al., 2015).

According to Haydon D.T. et al. (2002), vaccinating dogs against CDV is only effective in areas where they are a significant part of the CDV reservoir, thereby reducing the risk of infection. However, in Malaysia, due to deforestation and habitat loss, tigers are increasingly venturing into buffer zones and transition zones, increasing their contact with domestic dogs. In contrast, there is a lack of evidence that dogs are the reservoir, so the most likely way that tigers get infected was determined to be wild carnivores in Russia. In other regions, dogs are just one component of a larger and more complex reservoir community (Oleaga Á et al., 2022). In such cases, vaccinating free-roaming dogs alone may not be sufficient to completely prevent infections in tigers. (Gilbert M. *et al*., 2020).

The accessibility of labs with the necessary tests poses a challenge for CDV surveillance in wildlife globally. The process of obtaining permits (such as CITES) or restrictions on exporting samples can make it difficult to access foreign laboratories, resulting in delays in surveillance and discouraging field researchers from participating. To ensure sustainability and future surveillance, we decided to conduct the tests in Malaysia rather than exporting the samples. For this study, we utilized RT-PCR, a highly sensitive and specific technique that can accurately diagnose distemper in its early stages, regardless of the form of the disease, clinical symptoms, pathological observations, neutralizing antibody levels, or viral antigen distribution (Frisk et al., 1999). When using RT-PCR for early detection of CDV, conjunctival and nasal swabs are considered appropriate sample options (Nemeth

et al., 2018). The collection of swabs requires minimal staff training, time, and effort, and also reduces the risk of contamination. This can significantly increase the likelihood of detecting CDV and minimize the potential for errors.

In the future, further focused research is necessary to gather baseline data on Canine Distemper Virus (CDV) cases in wild carnivores in Malaysia. This will help us understand the contribution of domestic or feral animals to the local reservoir of CDV cases. Currently, the mode of transmission of the disease reservoir is unclear due to insufficient data on the risk of CDV in Malayan tigers. It is crucial to establish a disease surveillance and management strategy to effectively manage outbreaks and minimize future risks. Cooperation between WWF, other non-governmental organizations, and locals is essential in promptly investigating and containing CDV outbreaks. Additionally, the Wildlife and National Parks Department (PERHILITAN) should be notified of possible outbreaks. The Veterinary Services Department and local universities should also assist PERHILITAN in conducting further research to determine the prevalence of CDV.

6.0 CONCLUSION

In a nutshell, Canine Distemper Virus (CDV) is now emerging rapidly in wildlife. This study demonstrates the significance of ongoing wildlife monitoring and health surveillance in detecting emerging threats in endangered animals. It also provides an opportunity for researchers to develop and implement mitigation activities such as identifying CDV reservoir species and consideration and evaluation of vaccination strategies to reduce disease risk in Malayan tigers. Cooperation between the authorities, researchers, NGOs, and local communities is strongly encouraged to save the Malayan tigers.

7.0 LIMITATION AND RECOMMENDATION

One of the limitations of this study was that the sample size was too small to draw conclusions about the prevalence of CDV transmission for the whole large area. This is because we have limited time to capture more animals, as all captures are incidental and cannot be fully controlled by us. There are also limitations to the RT-PCR technique as it can only indicate the presence of viral material during infection. It does not indicate previous infection, level of immunity and antibody development.

It is recommended that a larger sample size and a longer sampling period be used to obtain more accurate prevalence data on CDV transmission in the area. Serological diagnosis, such as the serum neutralisation test (SNT), can be added to this study to determine past infection and the level of immunity and antibody development of the animals.

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APPENDIX

IBU PEJABAT
JABATAN PERLINDUNGAN HIDUPAN LIAR DAN
TAMAN NEGARA (PERHILITAN) SEMENANJUNG MALAYSIA
HEADQUARTERS
DEPARTMENT OF WILDLIFE AND NATIONAL PARKS (DWNP)
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Ruj. Kami: JPHL&TN (IP) 100-34/1.24 Jld 20(11)
Tarikh: 20 Mei 2022

Dr Tengku Rinalfi Putra Bin Tengku Azizan
Fakulti Perubatan Veterinar
Universiti Putra Malaysia
43400 UPM Serdang
SELANGOR

YBrs Dr,

KEPUTUSAN PERMOHONAN MENJALANKAN PENYELIDIKAN

Dengan hormatnya saya diarah merujuk kepada keputusan Mesyuarat Jawatankuasa Penyelidikan Jabatan PERHILITAN Bil.4/2022 bertarikh 1 April 2022 adalah berkaitan.

2. Sukacita dimaklumkan bahawa Jabatan **meluluskan** permohonan YBrs. Dr untuk menjalankan penyelidikan seperti butiran di bawah:

Nama Pemohon	:	**Dr Tengku Rinalfi Putra Tengku Azizan**
Rakan Saing		**Seperti di Lampiran A**
Institusi Pemohon	:	**Universiti Putra Malaysia**
Tajuk	:	***Canine Distemper Virus: Epidemiological and Molecular Genetics Studies on Domestic, Stray Dogs and Small and Medium Sized Wild Mammals and Its Threat to the Conservation of Malayan Tiger (Panthera tigris tigris Jacksonii)***
Lokasi & Spesies	:	***Seperti di lampiran B***
Tempoh kajian	:	**April 2022 - Mac 2024**

3. Sehubungan itu, YBrs Dr dipohon untuk melakukan beberapa perkara seperti berikut :

a. Rakan Saing & *co-author* Jabatan yang dilantik untuk memantau dan mengumpul laporan ialah Encik En. Mohd Lutfi Bin Abdullah & Puan Millawati binti Gani, Bahagian Konservasi Ex-Situ;

b. Mendaftar secara *online* permohonan Access and Benefit-sharing (https://www.myabs.gov.my/);

c. Duplikasi semua sampel hidupan liar yang diambil perlu diserahkan melalui rakan saing Jabatan;

d. Sebarang sampel tidak boleh dibawa ke luar negara;

e. Berkongsi hasil penyelidikan seperti laporan/penerbitan kertas saintifik/tesis dengan Jabatan melalui rakan saing;

f. Mengemukakan satu laporan hasil penyelidikan yang lengkap kepada Jabatan dalam tempoh dua (2) bulan selepas tamat penyelidikan;

g. Penyelidikan hendaklah diselesaikan dalam tempoh yang dinyatakan dalam permit; dan

h. Menghantar progress kajian kepada Jabatan melalui rakan saing setiap bulan Jun dan Disember dari tarikh kajian bermula.

4. Sebarang pertanyaan mengenai perkara ini, YBrs Dr boleh berhubung dengan Sekretariat Jawatankuasa Penyelidikan Jabatan PERHILITAN di talian 03-90866900 untuk maklumat lanjut. Segala perhatian dan kerjasama YBrs Dr dalam perkara ini didahului dengan ucapan terima kasih.

Sekian. Terima kasih

"WAWASAN KEMAKMURAN BERSAMA 2030"
"HIDUPAN LIAR UNTUK GENERASI AKAN DATANG"
"BERKHIDMAT UNTUK NEGARA"

Saya yang menjalankan amanah,

(DR. PAZIL BIN ABDUL PATAH)
Pengarah
Bahagian Konservasi Ex-Situ
b.p Ketua Pengarah
Jabatan Perlindungan Hidupan Liar dan
Taman Negara (PERHILITAN)

s.k.

Ketua Pengarah
Timbalan Ketua Pengarah (Konservasi)
Pengarah Bahagian Kawasan Perlindungan
Pengarah PERHILITAN Terengganu
Pengarah PERHILITAN Johor
Penguasa Taman Negara Pahang
Pengarah PERHILITAN Wilayah Persekutuan – Kaunter Permit

Encik Mohd Lutfi Bin Abdullah
Pegawai Penyelidik
Bahagian Konservasi Hidupan Liar

Puan Millawati binti Gani
Pegawai Penyelidik
Bahagian Konservasi Hidupan Liar