



**UNIVERSITI PUTRA MALAYSIA**

**DETECTION OF CANINE DISTEMPER VIRUS IN WILD TERRESTRIAL  
SMALL MAMMALS IN THE FOREST SURROUNDING FRUIT  
ORCHARDS OF BUKIT BESI, TERENGGANU**

**RAPHAEL CHAN YUI FUNG**

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SMALL MAMMALS IN THE FOREST SURROUNDING FRUIT ORCHARDS  
OF BUKIT BESI, TERENGGANU**

**RAPHAEL CHAN YUI FUNG**

A project paper submitted to the

Faculty of Veterinary Medicine, University Putra Malaysia

In partial fulfilment the requirement for the

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**FACULTY OF VETERINARY MEDICINE**

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## **CERTIFICATION**

It is hereby certified that we have read this project paper entitled “Detection of Canine Distemper Virus in Wild Terrestrial Small Mammals in the Forests Surrounding Fruit Orchards of Bukit Besi, Terengganu” by Raphael Chan Yui Fung and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement of the course VPD 4999-Project.

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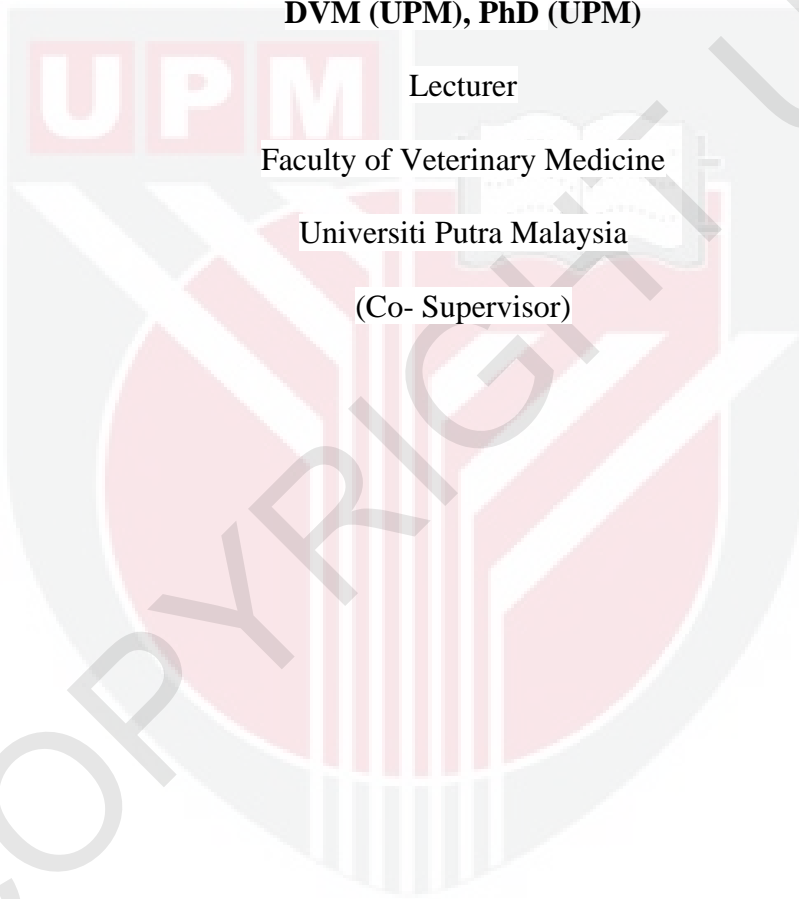
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**DEDICATION**

This thesis is dedicated to my supervisor, Dr. Tengku Rinalfi Putra Tengku Azizan, my co-supervisor, Dr. Khor Kuan Hua and Dr Wan Nor Fitri Wan Jaafar, my family, and friends.



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**LIST OF ABBREVIATIONS**

<b>CDV</b>	Canine distemper virus
<b>CNS</b>	Central nervous system
<b>RT-PCR</b>	Reverse transcription-polymerase chain reaction
<b>N</b>	Nucleocapsid
<b>P</b>	Phosphoprotein
<b>M</b>	Matrix protein
<b>F</b>	Fusion protein
<b>H</b>	Hemagglutinin
<b>L</b>	Large polymerase protein
<b>VNT</b>	Virus neutralization test
<b>ELISA</b>	Enzyme linked immunosorbent assay
<b>RNP</b>	Ribonucleoprotein complex
<b>CSF</b>	Cerebrospinal fluid
<b>IUCN</b>	International Union for Conservation of Nature
<b>SARS</b>	Severe Acute Respiratory Syndrome
<b>AUP</b>	Animal use protocol
<b>IACUC</b>	Institutional Animal Care and Use Committee
<b>RNA</b>	Ribonucleic acid
<b>cDNA</b>	Complementary deoxyribonucleic acid

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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 VIRUS CANINE DISTEMPER PADA MAMALIA KECIL  
TERRETRIAL LIAR DI HUTAN SEKELILINGI DUSUN BUAH BUKIT BESI,  
TERENGGANU**

Oleh

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**2023**

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Virus canine distemper (CDV) ialah virus RNA berantai tunggal yang sangat menular dan menimbulkan potensi risiko kepada pelbagai jenis spesies hidupan liar yang terdedah kepada virus itu. Sejak beberapa tahun kebelakangan ini, kematian harimau liar akibat virus canine distemper telah didokumenkan di Dungun, Terengganu. Tujuan kajian adalah untuk menyediakan data kehadiran CDV di kawasan tersebut dan menentukan kesan CDV

terhadap hidupan liar di Bukit Besi, Terengganu. Mamalia kecil darat liar di kawasan itu termasuk tikus padang Malaysia (*Rattus tiomanicus*), musang sawit Asia (*Paradoxurus hermaphroditus*), musang Malaya (*Viverra zibetha*). Kajian ini menyaring mamalia kecil darat liar untuk CDV menggunakan ujian tindak balas rantai transkripsi-polimerase terbalik (RT-PCR). Mamalia kecil itu ditangkap menggunakan perangkap sangkar yang diletakkan di dalam hutan mengelilingi dusun buah Bukit Besi. Mamalia kecil yang ditangkap telah dibius, dan kemudian sampel swab hidung dan konjunktiva diperolehi. Kedua-dua sampel dikumpulkan, disimpan dalam 4°C dan diangkut ke makmal virologi. Sampel diuji menggunakan ujian RT-PCR. Sebanyak 11 ekor tikus padang, 3 ekor musang sawit Asia dan 1 ekor musang Malaya berjaya ditangkap. Sampel swab daripada kesemua 15 haiwan yang ditangkap telah diuji. Disimpulkan bahawa haiwan yang ditangkap dalam kajian ini tidak dijangkiti CDV. Walau bagaimanapun, saringan CDV dalam hidupan liar akan diteruskan untuk memantau status CDV di hutan Bukit Besi dan untuk merangka pelan tindakan yang diperlukan untuk mencegah wabak CDV, yang merupakan potensi ancaman kepada spesies hidupan liar dan terutamanya harimau liar yang terancam.

Kata kunci: *CDV; harimau liar; RT-PCR; mamalia kecil darat liar; status CDV*

**ABSTRACT**

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

**DETECTION OF CANINE DISTEMPER VIRUS IN WILD TERRESTRIAL  
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**By**

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**2023**

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Canine distemper virus (CDV) is a single-stranded RNA virus that is highly contagious and poses a potential risk to a wide variety of wildlife species that are susceptible to the virus. In recent years, the death of wild tigers due to canine distemper virus has been documented in Dungun, Terengganu. The purpose of the study was to provide data on the presence of CDV in the area and determine the impact of CDV on the wildlife in Bukit Besi, Terengganu. Wild terrestrial small mammals in the area include Malaysian field rat

(*Rattus tiomanicus*), Asian palm civet (*Paradoxurus hermaphroditus*), Malayan civet (*Viverra zibetha*). This study screened the wild terrestrial small mammals for CDV using reverse transcription-polymerase chain reaction (RT-PCR) test. The small mammals were captured using cage traps placed in the forest surrounding the fruit orchard of Bukit Besi. The captured small mammals were anaesthetized, and then nasal and conjunctival swab samples were obtained. Both samples were pooled, stored in 4°C and transported to the virology laboratory. Sample was tested using RT-PCR test. A total of 11 field rats, 3 Asian palm civets and 1 Malayan civet were captured. The swab samples from all 15 captured animals were tested. It was concluded that the animals captured in this study were not infected with CDV. However, the screening of the CDV in wildlife will be continued to monitor the CDV status in the forests of Bukit Besi and to formulate necessary action plans to prevent the CDV outbreak, which is a potential threat to the wildlife species and especially the endangered wild tigers.

Keywords: *CDV; wild tigers; RT-PCR; wild terrestrial small mammals; CDV status*

## 1.0 INTRODUCTION

Canine Distemper Virus (CDV) is a single-stranded RNA virus of the genus Morbillivirus in the Paramyxoviridae family (Griffin, 2001). It is a highly contagious virus that has a worldwide distribution. The virus is mainly found in dogs, but it can also infect a wide range of mammalian families such as Canidae, Felidae, Mustelidae, Viverridae, Muridae and so on (Martinez-Gutierrez and Ruiz-Saenz, 2016). CDV was initially documented as a disease impacting dogs in the Quito region and other parts of South America in 1746 (Blancou, 2004). Its presence was later noted in Spain in 1760, resulting in a significant outbreak with 900 deaths reported in one day in Madrid, the virus then spread to Great Britain and Italy in the ensuing years (Blancou, 2004). The common symptoms of CDV that are shown in domestic dogs are anorexia, fever, and upper respiratory clinical signs. In tigers, the observed clinical signs include respiratory, ocular, gastrointestinal, and CNS signs such as seizures, ataxia, myoclonus, and coma (Appel et al., 1994). The predominant mean of transmission involves direct contact between a vulnerable dog and an infected dog or other wildlife animal. CDV primarily spreads through the aerosolization of respiratory exudate and bodily excretions like urine from an infected host. Shedding of CDV may persist for a period of 60 days to 90 days following an infection (Greene et al., 1990).

Malayan tigers (*Panthera tigris jacksoni*) are the tiger species native to Peninsular Malaysia. They are mainly distributed in the peninsular of Malaysia in

Pahang, Kelantan, Terengganu, and Perak (Kawanishi et al., 2003). The body length of female tigers ranged from 180 cm to 260 cm and their body weight ranged from 24 kg to 88 kg (Khan, 1998). The males have a body length ranged from 190 cm to 280 cm and a body weight ranged from 47.2 kg to 129.1 kg (Khan, 1998). Malayan tigers are great swimmers and love to spend their time in the water. They are nocturnal animals that sleep or travel in the daytime and usually hunt at night. As an apex predator, they hunt on various species of animals in the forests of Malaysia that nurture a rich biodiversity. These territorial, solitary animals require a large territory and other tigers of the same sex are not allowed to enter the territory to ensure enough food source and mating partners for their continuity. However, human activities such as logging, agricultural expansion and urbanization lead to deforestation which reduces the habitat and the population of Malayan tigers. The Malayan tigers had been classified as critically endangered on the IUCN Red List since 2015. The population of Malayan tigers were decreasing yearly and there less than 150 tigers left in Malaysia currently. It is detrimental to the rainforest of Malaysia as tigers have a very vital ecological importance to the food chain. Tigers act as the tertiary consumer and are needed to control the populations of the herbivores and omnivores such as boars, deer, gaurs and so on. This helps to ensure the plants are in appropriate amount to maintain the ecological balance.

Recent years, two cases of tiger death due to canine distemper virus infection that happened in Terengganu and Johor, Malaysia raised the concern about the threat that canine distemper virus posed to the Malayan tigers. According to The Sun

Newspaper, the tame male tiger from Dungun, Terengganu named 'Awang Besul' was reported died in 23<sup>rd</sup> of July 2019 due to canine distemper virus infection. A study in Russia had identified CDV as a risk to Amur tigers (*Panthera tigris altaica*) and it could increase the probability extinction of the tigers in 50 years by 65% (Bodgener et al., 2023). Deforestation, poaching, urbanization, and other factors greatly contributed to the crisis of decreasing numbers of Malayan tigers and the canine distemper virus will only further worsen the problem. Thus, necessary prevention plans must be formulated before canine distemper virus emerges as a huge problem in the survival of Malayan tigers. However, the data about the canine distemper status in Malaysia was limited and it was needed for the authorities to assess the condition and take actions. By carrying out the study, we can have a clearer view about the canine distemper virus status and have better preparation for any worse situation such as CDV outbreak.

Wild terrestrial small mammals are small wildlife mammals that spend most of their time carrying out daily activities mainly on the ground. Some of the examples of wild terrestrial small mammals are field rats, civets, mousedeer, wild cats, porcupines and so on. In this study, the mammals that were mainly focused on was Malaysian field rat (*Rattus tiomanicus*), followed by Asian palm civet (*Paradoxurus hermaphroditus*) and Malayan civet (*Viverra zibetha*).

Malaysian field rat (*Rattus tiomanicus*), also known as Malaysian wood rat, is a rodent under the Muridae family. It is widely distributed in Malaysia, Thailand, Indonesia, Philippines, Singapore. The body length of Malaysian field rat ranged from

80 mm to 160 mm. It has a long tail that is covered with thin fur and the tail length is between 85 mm to 170 mm (Liat, 2015). The body weight of the field rat ranged from 55 g to 150 g. It has smooth fur around the whole body except for the ear and footpad. The fur at the dorsal part of the body is brown in color while the fur at the ventral part of the body is pure white or yellowish white. These rodents live as scattered communities in the forests near plantation areas and they are known as pests to the villagers. As a nocturnal omnivore, the field rats rest on the trees during the day and forage on the ground at night. The Malaysian field rat have a wide range of diet from insects to plantation crop and includes food scrap from the residential area. There are different kinds of rat borne disease which are transmissible to other animals and humans. Rats may carry different kinds of pathogen such as virus, bacteria and parasite which may cause disease transmission to animals and humans. Some examples of rodent borne diseases are Hantavirus pulmonary syndrome, Lymphocytic Choriomeningitis virus (LCMV) infection, Lyme disease, Plague caused by bacteria *Yersinia pestis*, Leptospirosis and other parasitic disease. (Meerburg et al., 2009)

Asian Palm Civet (*Paradoxurus hermaphroditus*) is also known as common palm civet and musang. The Asian palm civet is widely distributed in countries of Southeast Asia such as Malaysia, Singapore, Brunei Darussalam, Thailand, India, Nepal, Bangladesh, Bhutan, Myanmar, Sri Lanka, Laos, Cambodia, Vietnam, China, Philippines, and the Indonesian islands (Duckworth, 2011). They have grey, coarse hair coat with black markings on the body with a long and un-ringed tail. The muzzle, ears, lower legs, distal half of the tail are black. It is also a Solitary and nocturnal omnivore

that lives in the trees and on the ground. As a solitary animal, it is active at night and rest in the morning. It is both terrestrial and arboreal animal, forages for food on the ground and in the tress. The diet includes fruits such as berries and pulpy fruits, flower sap, rats, birds, insect, and invertebrate. There are studies showed that Asian palm civets are susceptible to CDV. According to Techangamsuwan et al. (2014), CDV quickly killed 18 Asian palm civets, a masked palm civet and a small Indian civet in Thailand. All civets were tested with the commercial canine distemper virus (CDV) antigen test kit, and all showed positive result. Besides, Asian palm civets were also involved in the transmission of zoonotic disease such as SARS. The epidemic emerged in 2003 in Guangdong, China after the waitress that served palm civets as food and a customer who ate it near the animal cages at a restaurant in Guangzhou, China were diagnosed for SARS. (Wang et al., 2005). All 6 palm civets at the restaurant were tested positive for SARS-associated coronavirus (Wang et al., 2005).

Malayan civet (*Viverra zibetha*) is also known as the Malay civet or Oriental civet. The distribution is mainly at Peninsular Malaysia, Borneo, Philippines, Island of Indonesia such as Sumatra and Riau (Corbet & Hill, 1992). It can be easily recognized by the black color ring or band on its tail. It has numerous dark spots on the flanks and a dark line along the back that extends to the tail. As a solitary and nocturnal omnivore, it mostly forages for food on the ground (Kanchanasakha et al., 1998).

The location for sampling was done at the forest surrounding orchards of Agro Exotic farm in a village of Bukit Besi known as Kampung Besul. It was in Dungun,

Terengganu. Kampung Besul was chosen as the sampling site as it was one of the places that was reported with the sighting of a tame wild tiger, which was tested positive of canine distemper virus infection after it died. The forest contained several types of wild terrestrial small mammals, the most common animal in the area is Malaysian field rat (*Rattus tiomanicus*). Asian palm civet (*Paradoxurus hermaphroditus*) and Malayan civet (*Viverra zibellina*) are also wild terrestrial small mammals that were captured in the location.

### **1.1 OBJECTIVE**

Overall objective: To screen wild terrestrial small mammals for CDV in habitat biota of Malayan tiger in forest of Bukit Besi.

Specific objective: To test the efficacy of RT-PCR test using nasal and conjunctival swab as a method for detection of CDV in wild terrestrial small mammals in forest surrounding fruit orchard of Bukit Besi.

### **1.2 HYPOTHESIS**

Null hypothesis ( $H_0$ ): There is no presence of CDV infections in wild terrestrial small mammals in the forest surrounding fruit orchard of Bukit Besi, Terengganu.

Alternative hypothesis ( $H_A$ ): There is presence of CDV infections in wild terrestrial small mammals in the forest surrounding fruit orchard of Bukit Besi, Terengganu.

## **2.0 LITERATURE REVIEW**

### **2.1 CANINE DISTEMPER VIRUS (CDV)**

CDV is a non-segmented single negative-stranded RNA virus under the genus Morbillivirus in the Paramyxoviridae family with a lipoprotein envelope and the size ranged around 150 - 250 nm (Greene and apple, 1990). The CDV genome structure is characterized by six transcription units (N-P-M-F-H-L) arranged linearly (Kolakofsky, 2016). The six units are Nucleocapsid (N), Phosphoprotein(P), Matrix protein (M), Fusion protein (F), Hemagglutinin (H), Large polymerase protein (L). The genomic RNA is enveloped by the nucleocapsid (N) protein. During CDV gene expression, N serves as a template for the transcription and replication involving the viral polymerase (L) and its cofactor, the phosphoprotein (P) (von Messling et al., 2001). Together with the viral RNA, the N, P, and L proteins constitute the ribonucleoprotein (RNP) complex. The envelope of CDV comprises two integral membrane proteins, which are the fusion (F) and hemagglutinin (H) proteins. The membrane-associated protein M plays a role in facilitating contact with the ribonucleoprotein (RNP) during the budding process in the host cell membrane, completing the formation of the viral envelope (da Fontoura, 2016). H glycoprotein facilitates the binding of the virus to the host cell membrane, while the F protein accomplishes the fusion of the viral and host membranes (von Messling et al., 2001). This fusion process allows the entry of the viral ribonucleoprotein (RNP) into the host cell cytoplasm.

## **2.2 SOURCE AND TRANSMISSION**

The predominant mode of CDV transmission is through the respiratory tract, either through aerosol or saliva, particularly during close contact with an infected individual. Transmission between different species is also thought to take place through predation. Gilbert et al. (2015) also stated that CDV transmission can occur when tigers prey on the host species that were infected by CDV. Besides, contact with infectious material, such as urine or feces, provides other avenues for transmission. The virus is shed through body fluids such as nasal secretions, ocular secretions, saliva and urine. The CDV is extremely contagious, and shedding of the virus may persist for a period of 60 to 90 days following infection (Greene et al., 1990). In captive tigers, the virus has been identified in the urine for up to 150 days following infection (Gilbert et al., 2015).

## **2.3 PATHOGENESIS AND CLINICAL SIGNS**

The pathogenesis of CDV in domestic dogs has been thoroughly characterized, and it may exhibit similarities in non-domestic species. As the virus enters through the epithelium of the upper respiratory tract, it undergoes multiplication in macrophages, and then disseminates to the tonsils and regional lymph nodes. After the initial replication in the tonsils, the virus circulates throughout the body via the lymphatic and blood systems (Ludlow et al., 2013). During the early viremic phase, the virus caused leukopenia and immunosuppression leading to transient fever in the animal. The second

phase usually manifests around 8-9 days after infection, and animals begin to exhibit noticeable symptoms during the period (Sykes, 2014). Through a cell receptor known as nectin-4, the virus can infect a wider array of cells, encompassing epithelial cells found in the gastrointestinal and respiratory mucosa (Noyce et al., 2013). This leads to the presentation of gastrointestinal and respiratory signs in the infected animal. The final phase of infection is when the CDV proceeds to attack the CNS causing neurological signs. Viruses are present in the cerebrospinal fluid and CSF is a suitable sample for diagnosis in this stage.

The clinical signs in dogs are associated with upper respiratory signs, gastrointestinal signs and neurological signs. Fever, anorexia, dehydration, conjunctivitis, pneumonia, diarrhea, and infections secondary to leukopenia, are commonly shown in CDV infected animals (Williams, 2001). Neurological signs include ataxia, seizure, myoclonus, hyperesthesia, cervical rigidity, cerebellar and vestibular signs, and paraparesis. Tigers in captivity show respiratory signs like ocular and nasal discharge, gastrointestinal signs such as anorexia, vomiting and diarrhea (Appel et al., 1994). CDV infected tigers in the wild usually show neurological signs such as loss fear to human or roaming close to the living area of human. Neurological signs like seizures, ataxia, myoclonus, and coma before death were also noticed in the captivated tigers (Appel et al., 1994).

## 2.4 DIAGNOSIS

Laboratory diagnosis of CDV includes RT-PCR, VNT, ELISA, immunochromatographic assays. RT-PCR has a high sensitivity which makes it capable of detecting the virus in the sample regardless of the amount. Samples from body tissues and body fluids such as nasal and ocular secretions, urine, CSF, saliva, blood and serum can be used for the RT-PCR test. Body tissue should be sampled based on the location of the CDV lesion. Enzyme-linked immunosorbent assays (ELISA) have been created for the detection of serum IgG and IgM antibodies to CDV (Von Messling et al., 1999). Nevertheless, these tests depend on secondary marker antibodies that are species-specific. This specificity implies that tests developed for dogs are only dependable for detecting antibodies in dog serum and it may not be useful for other wildlife species. Immunochromatographic assays are normally used in rapid detection kits for a fast result. Body fluids of the suspected animals are used as the sample. Antibody detection kit detects for CDV antibodies in the serum or plasma to check for past exposure while antigen detection kit detects for CDV in the sample to examine for active infection. Virus neutralization test (VNT) is an appropriate procedure that can provide a definitive diagnosis of CDV. However, it requires cell culture techniques and the capability of tested serum samples to neutralize live viruses which is a complicated procedure that necessitates a significant amount of time. Physical examination such as the respiratory,

gastrointestinal and neurological signs, blood result can be used to support the laboratory diagnosis.

## **2.5 TREATMENT AND PREVENTION**

There is no treatment for the disease, only palliative treatment was available for the infected animals. Good nursing care, rehydration fluids, sufficient nutrition are important for boosting the immune system for recovery. Antibiotics and anticonvulsant can be given when infection and neurological signs are involved.

Prevention can be achieved by administering vaccine to the susceptible animals. Several vaccines for canine distemper are accessible for dogs and other domestic as well as non-domestic animals (Deem et al., 2000).

### **3.0 MATERIALS AND METHODS**

#### **3.1 ANIMAL ETHICS**

The animal capturing was conducted under the supervision of the veterinarian and in accordance 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-U040/2023.

#### **3.2 ANIMAL CAPTURING AND SAMPLING**

The animal capturing was done at the forest area surrounding the agro farm in Kampung Besul, Bukit Besi, Terengganu. Cage traps were placed at different locations of the forest mostly at the margin of the forest near the tarred road. Food baits such as banana and betel nuts were placed in the cage traps to attract the targeted animals. The cage traps were placed for a night and were checked in the next morning. Captured animals were properly restrained for the injection of anaesthetic drug through intramuscular injection at the quadriceps muscle. Ketamine and xylazine were used as the anaesthetic drug, the concentration for both drugs were 100 mg/mL. For rats, 0.2 mL of ketamine was given with 0.04 mL of xylazine and for civets, 0.7-0.8 mL of ketamine was given with 0.06 mL of xylazine.

After the animal was completely unconscious, nasal and conjunctival swab samples were obtained using the cotton swab stick. The lower eyelid was pulled away to expose the conjunctival membrane. The swab was gently rubbed at the conjunctival membrane until it was moist. The swab stick was inserted into the sterile tube with the universal virus transport medium and was snapped to ensure the tip of the swab stick entered the transport medium. The swab stick should not be contaminated by the environment. Nasal swab was taken by inserting the cotton swab stick into the nares as deep as possible. The swab stick was rotated carefully in a circular motion to ensure it was properly covered by the nasal secretions. The swab stick for nasal swabbing was placed together with the conjunctival swab in the transport media as a pool sample. The tube was then stored under 4 degrees Celsius with the ice pack. The animals were contained in a captivity with food and water supply for one night to observe for any side effects or complications from the anaesthesia. The confinement was done under the animal care and use following the guidelines in the Institutional Animal Care and Use Committee (IACUC) form. The animals were released back into the wild after they recovered from the anaesthesia in the next morning.

### **3.3 2-STEP RT-PCR TEST**

The samples were sent to virology lab to undergo RT-PCR test following the NucleoSpin® RNA Virus protocols. (MACHEREY-NAGEL GmbH & Co. KG, 2020).

The first step is to lyse the viruses.

1. 600  $\mu$ L Buffer RAV1 containing Carrier RNA was added to 150  $\mu$ L of the sample.

The mixture was pipetted up and down and vortexed well. It was then incubated for 5 minutes at a water bath of 70 °C. The mixture was centrifuged for 1 minute at 11,000 times gravity and the supernatant was removed to prevent clogging of the NucleoSpin® RNA Virus Column.

The next step was to adjust the binding conditions of the viral RNA.

2. 600  $\mu$ L of ethanol with the concentration from 96% - 100 % was added to the clear lysis solution and the solution was vortexed for 10 - 15 seconds to thoroughly mix it.

The next procedure was to bind the viral RNA.

3. The NucleoSpin® RNA Virus Columns was placed in the 2mL collection tubes and 700  $\mu$ L lysed sample was loaded. The solution was centrifuged for 1 minute at 8,000 times gravity and the residual lysis solution was loaded onto the NucleoSpin® RNA Virus Column. It was then centrifuged for 1 minute at 8,000 times gravity. The flowthrough was discarded and the NucleoSpin® RNA Virus Column was placed into another new 2mL collection tube. (More than two loading steps are not recommended.)

The next step was to wash and dry the silica membrane for 3 times.

4. In the first wash, 500  $\mu\text{L}$  of Buffer RAW was added to the NucleoSpin® RNA Virus Column. It was then centrifuged for 1 minute at 8,000 times gravity, and the flowthrough was discarded. This step in the washing process removed contaminants and PCR inhibitors.
5. For the second wash, 600  $\mu\text{L}$  of Buffer RAV3 was added to the NucleoSpin® RNA Virus Column, followed by centrifugation for 1 minute at 8,000 times gravity. The flowthrough, along with the collection tube was discarded.
6. In the third wash, the NucleoSpin® RNA Virus Column was placed in a new 2 mL collection tube, and 200  $\mu\text{L}$  of Buffer RAV3 was added. Centrifugation for 2 - 5 minutes at 11,000 times gravity to effectively remove the ethanolic Buffer RAV3.

The next procedure was to elute the viral RNA.

7. The NucleoSpin® RNA Virus Column was placed into a new and sterile 1.5 mL microcentrifuge tube. 50  $\mu\text{L}$  of RNase-free H<sub>2</sub>O was preheated to 70 °C and added, the solution was incubated for 1–2 minutes. Centrifugation was carried out for 1 minute at 11,000 times gravity.

Next step was to synthesize cDNA from the viral RNA following the SensiFAST cDNA Synthesis Kit Protocol. (Meridian Life Science Inc, USA. SensiFAST cDNA Synthesis Kit Protocol).

8. Master mix solution for the cDNA synthesis was created by mixing total RNA or mRNA with TransAmp Buffer, Reverse Transcriptase and DNase/RNase free

water. The solutions were vortexed and centrifuged before mixing. The mixing was done by pipetting gently.

9. The thermal cycler was set at 25 °C for 10 minutes to undergo primer annealing. The temperature was then increased to 42 °C for 15 minutes for the reverse transcription process. Inactivation was completed at 85 °C for 5 minutes. Lastly, the temperature was reduced and held at 4 °C.

The next step is to amplify the resulting cDNA with the PCR thermal cycler following the Standard MyTaq Red Mix Protocol (Meridian Life Science Inc, USA. MyTaq™ Red Mix).

10. The master mix solution was prepared by mixing the Template, Primers, MyTaq Red Mix and Water (ddH<sub>2</sub>O).
11. After the initial denaturation was done in the thermal cycler, denaturation, annealing and extension process was done at 25 to 35 cycles.

The last step was to place the PCR product to the gel for electrophoresis and view the result.

12. The agar gel was prepared with buffer solution and agarose gel powder.
13. DNA ladder and PCR products were pipetted into the agar gel.
14. The electrophoresis was done at 90V and 350mA for at least 25 minutes.
15. The result was observed under the computer.

#### 4.0 RESULT

The study screened for the occurrence of Canine distemper virus among wild terrestrial small mammals in Kampung Besul, Bukit Besi.

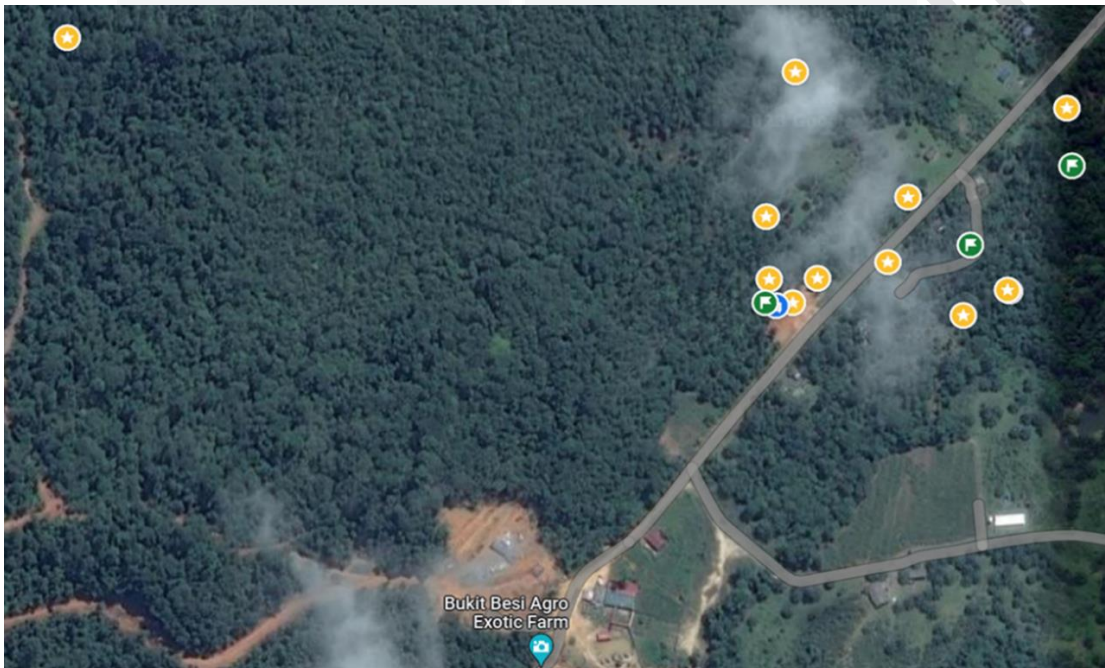


Figure 1 shows the exact location where the animals were caught.

The yellow symbol represents the capturing location of the field rats, the green symbol represents the capturing location of the Asian palm civet, blue symbol represents the capturing location of the Malayan civets. A total of 17 animals were captured in the study. Among the captured animals, 73.33% were field rats (11/15), 20% were Asian palm civets (3/15) and 6.67% were Malayan civet (1/15). The RT-PCR test was done and all the swab samples from the 15 animals showed negative results.

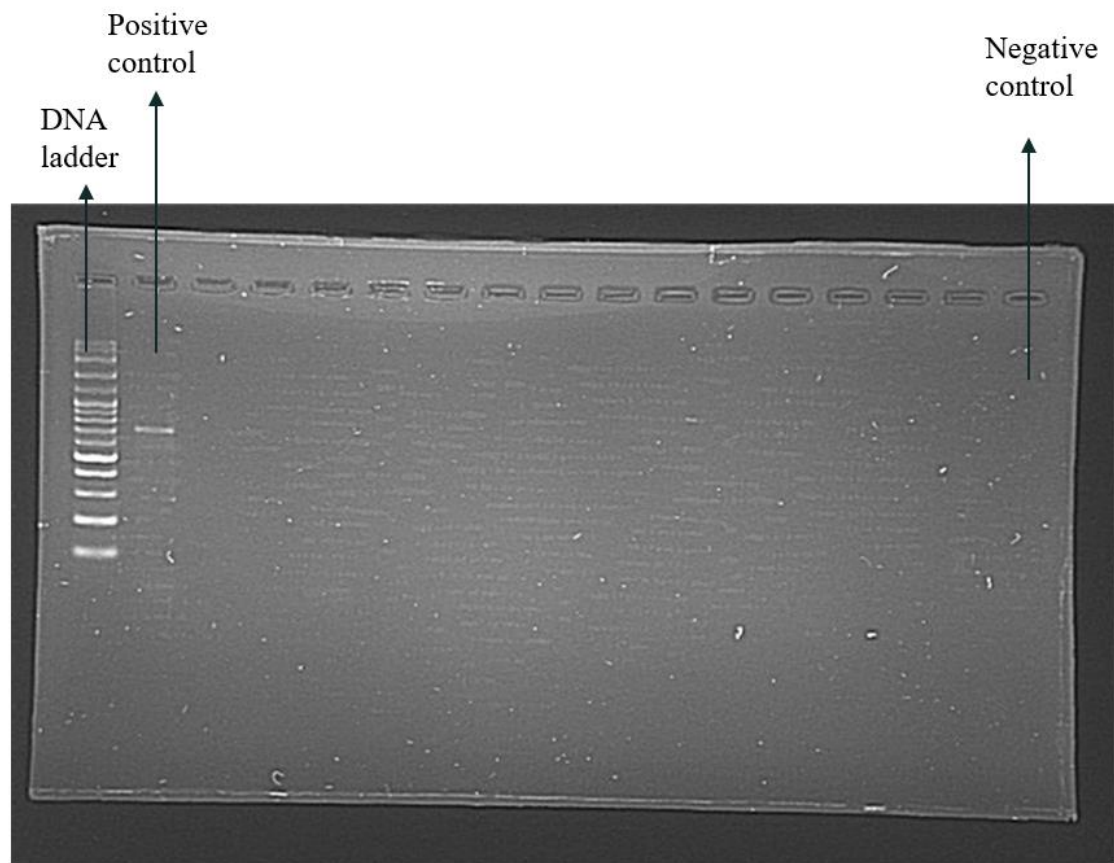


Figure 2 shows the result of the RT-PCR test.

The PCR result for all the 15 animals was negative.

## 5.0 DISCUSSION

Malaysian field rats (*Rattus tiomanicus*) were focused on this study and were mainly sampled in the detection of CDV in small terrestrial wild mammals. As the field rats are the most populated small terrestrial wild mammals in the area and are playing an important role in transmitting diseases. CDV was transmitted from the rats to the wildlife in the forests of Bukit Besi and finally transmitted to the tigers. The transmission of CDV to the wildlife and local dogs occurred through direct contact and indirect contact from the rats. The possible transmission route is that a CDV-infected rat sheds the virus through body fluids such as urine in the village area when it searches for food. Local dogs or other wildlife that roamed in the village area were exposed to the infected rat and its body fluid and got infected. The infected wild animals then returned to the deep part of the forest, continued transmitting CDV to other animals and exposed the disease to the tigers. Due to deforestation and loss of habitat, wild tigers may also search for food at the margin of the forest which is near the village area. The wild tigers will have contact with the local dogs, exposing themselves to CDV infection. Thus, detection results from the rats greatly contributed to the CDV infection status in Bukit Besi.

RT-PCR test was chosen as the method of detection for the CDV. RT-PCR tests are highly effective in identifying the presence of a pathogen or its derivatives, and they are a very useful method for detecting an ongoing infection. Throughout the CDV

infection, the virus can be detected in various bodily fluids such as nasal secretions, conjunctival secretions, cerebrospinal fluid, and urine. The advantages of the RT-PCR test are the high sensitivity of the test, and it can detect small fragments of genetic materials and amplify it for detection. However, the transient nature of viral shedding from different sites at different stages of the disease requires caution when interpreting results. Positive results from RT-PCR assays should generally be considered true positives unless there is evidence of contamination. This molecular technique allows for identifying of the nucleoprotein (NP) gene associated with the virus (Shin et al., 1995), providing a sensitive means of confirming the presence of CDV.

The negative RT-PCR result was attributed to several reasons which included the limitation of the RT-PCR test. RT-PCR tests cannot be used to detect past infections. Shedding of Canine distemper virus only occurs for a specific window of time for 60 to 90 days. (Greene & Appel, 1990). RT-PCR is most effective during the active phase of infection when the CDV genetic material is replicating. However, after the shedding period of CDV has passed, the CDV will not be present in the body fluids of that animal and RT-PCR cannot detect the NP gene of CDV. The negative result may be that some of the animals had recovered from the previous infection and stopped shedding the virus. In addition, the number of animals in this study is too few compared to the total population in Bukit Besi. In this study, only 15 animals were included for the CDV detection and many more animals living in the area. So, the result in this study may have limited similarity to the real situation of the population in Bukit Besi. More animal samples must be included to have a more reliable and accurate conclusion. Lastly, the

captured animals are mainly from the northeast area of the agro farm, and there is a possibility that the sampling area had a lower CDV infection status than the other parts of the forest. Another possible reason is that the CDV infection status in Bukit Besi is still low, making it challenging to capture an infected animal.

Other samples for the RT-PCR test such as whole blood, serum, urine, vaginal smears and tracheal washing can be taken as swab samples for detection. According to Tipold et al. (1992), the conclusive antemortem diagnosis of distemper relies on the detection of viral antigens in scrapings and various body fluids, including conjunctival and vaginal smears, tracheal washings, and urine sediment. The study from Frisk et al. (1999) demonstrated that serum and whole blood samples were used for RT-PCR test, but negative results were observed in affected dogs presenting nervous deficits. Conversely, serum and whole blood samples from distemper-affected dogs displaying gastrointestinal or respiratory signs exhibited a high sensitivity for CDV using RT-PCR (Frisk et al., 1999).

One of the limitations of RT-PCR is that it does not provide information about the immune response or the presence of antibodies. Serological tests such as immunochromatographic assays and VNT can be included to detect the previous infection in the animals for a more accurate CDV infection status. Goodrich et al., (2012) also stated that serological assays are the most appropriate technique for conducting routine surveillance or investigating population-level exposure.

As digital hyperkeratosis or hard pads are one of the clinical signs shown in the affected dogs (Gelatt et al.,1985), paw swab might be a useful sample for detection of CDV through RT-PCR test. As the procedure is less invasive compared to other swabbing method, it may be used for sampling of wild animals as it is less stressful to the animals. This may reduce the usage of anaesthetic drugs and further reduce the possible complications of the sampled animals from anaesthesia or restraining stress. Thus, more research can be done based on the paw swab method to examine the reliability and if the method is useful, it will ease the sampling of the wild animals.

## **6.0 CONCLUSION**

As a conclusion, none of the animals in this study are infected with CDV.

However, the screening of CDV will be continued to monitor the cdv status and provide the data for us to have a clearer view of the situation. Having more understanding of the situation in the forest of Terengganu can help the authorities to formulate necessary action plans to protect the tigers. Continuous CDV surveillance can also help to prevent a CDV outbreak by sharing the surveillance information with all the related parties.

## **7.0 LIMITATIONS AND IMPROVEMENTS**

The limitation of this study is the low number of samples which might affect the accuracy of the result. More animal samples must be collected in different areas of Bukit Besi to improve the accuracy and validity of this study. To have a clearer view of the CDV infection status in wild terrestrial small mammals in Bukit Besi, at least 200 animals must be sampled. As the cage trapping of animals is opportunistic, more time should be allocated for the sampling of animals to obtain a larger sample size.

Another limitation is that RT-PCR test requires laboratory and laboratory personnel to undergo complicated procedures. The samples must be sent to the laboratory from the field, and it takes a long time to complete the laboratory procedures to obtain the result. Due to these reasons, RT-PCR test takes a long time to provide the result. Thus, a CDV test kit can be used to obtain the result in a much shorter time on the field. This can help to save more time and manpower resources which can be used on the field sampling instead.

## 8.0 REFERENCE

Appel, M. J., Yates, R. A., Foley, G. L., Bernstein, J. J., Santinelli, S., Spelman, L. H.,

Miller, L. D., Arp, L. H., Anderson, M., & Barr, M. (1994). Canine distemper epizootic in lions, tigers, and leopards in North America. *Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, 6(3), 277–288.

Barret T. & Mahy, B. V. R. M. (2010) Rinder pest and distemper viruses. *Desk encyclopedia of animal and bacterial virology*. San Diego; 221–231.

Blancou J. (2004). Dog distemper: imported into Europe from South America? *Hist. Med. Vet.* 29, 35–41.

Bodgener, J., Sadaula, A., Thapa, P.J., Shrestha, B.K., Gairhe, K.P., Subedi, S., Rijal,

K.R., Pandey, P., Joshi, J.D., Kandel, P., Lamichane, B.R., Pokheral, C.P.,

Subedi, N., Kandel, R.C., Luitel, H., Techakriengkrai, N. and Gilbert, M. (2023):

Canine distemper virus in Tigers (*Panthera tigris*) and leopards (*P. Pardus*) in Nepal. 12(2): 203.

Corbet, G. B. & Hill, J. E. (1992). Mammals of the Indomalayan region. A systematic review. Oxford: Oxford University Press.

da Fontoura Budaszewski, R., & von Messling, V. (2016). Morbillivirus Experimental Animal Models: Measles Virus Pathogenesis Insights from Canine Distemper Virus. *Viruses*, 8(10), 274. <https://doi.org/10.3390/v8100274>

Deem, S. L., Spelman, L. H., Yates, R. A., & Montali, R. J. (2000). Canine distemper in terrestrial carnivores: a review. *Journal of zoo and wildlife medicine: official publication of the American Association of Zoo Veterinarians*, 31(4), 441–451.

Duckworth, J. W., Widmann, P., Custodio, C., Gonzalez, J., Jennings, A. and Veron, G. (2011). "Paradoxurus hermaphroditus" (On-line). The IUCN Red List of Threatened Species.

Frisk, A. L., König, M., Moritz, A., & Baumgärtner, W. (1999). Detection of canine distemper virus nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. *Journal of clinical microbiology*, 37(11), 3634–3643.

<https://doi.org/10.1128/JCM.37.11.3634-3643.19994>

Gelatt, K.N, D. Whitley, D. A. Samuelson, and Garcia-Sanchez., G. (1985): Ocular manifestations of viral diseases in small animals. *Compend Contin. Educ. Pract. Vet.* 7: 968–976.

Gilbert, M., Soutrina, S., Seryodkin, I., Sulikhan, N., Uphyrkina, O., Goncharuk, M., Matthews, L., Cleaveland, S. and Miquelle, D. (2015). Canine distemper virus as a threat to wild tigers in Russia and across their range. *Integrative Zoology*. 10(4): 329-343.

Goodrich, J.M., Quigley, K.S., Lewis, J.C., Astafiev, A.A., Slabi, E.V., Miquelle, D.G, Smirnov, E.N., Kerley, L.L., Armstrong, D.L., Quigley, H.B., and Hornocker, MG. (2012). Serosurvey of free-ranging Amur tigers in the Russian Far East. *Journal of Wildlife Diseases*. 48(1):186-189.

Greene, G. E., and M. J. Appel. (1990). Canine distemper. *Infectious Diseases of the Dog and Cat*. W. B. Saunders, Philadelphia, Pennsylvania. 226–241.

Kanchanasakha, B., Simcharoen, S. and Tin Than, U. (1998). Carnivores of Mainland South-East Asia. Endangered Species Unit, WWF-Thailand Project Office, Thailand.

Kawanishi K., Yatim, S. H., Abu Hashim, A. K. and Topani, R. (2003). Distribution and potential population size of the tiger in peninsular Malaysia. *J Wildl Parks (Malaysia)* 21:29-50.

Khan, M.K.M. (1986). "Tigers in Malaysia". *The Journal of Wildlife and Parks*. V: 1–23.

Kolakofsky D. (2016). Paramyxovirus RNA synthesis, mRNA editing, and genome hexamer phase: a review. *Virology*;498:94–8.

Liat, L. B. (2015). The field rats and field mouse in Malaysia and Southeast Asia. *UTAR AGRICULTURE SCIENCE JOURNAL*, 1(3), 35–42.

Lim, N. T. L. and Ou Yang, X. (2012). Occurrence of the malay civet, *Viverra zangalunga* (Mammalia: Carnivora: Viverridae) in Singapore. *Nature in Singapore*. 5: 79–81.

Ludlow, M., Rennick, L., Nambulli, S., de Swart, R. & Duprex, W. (2013). Using the ferret model to study morbillivirus entry, spread, transmission and cross-species infection. *Current Opinion in Virology*, 4C: 15-23.

MACHEREY-NAGEL GmbH & Co. KG (2020). Viral RNA isolation User manual.

NucleoSpin® RNA Virus. Takara Bio USA, Inc.

[https://www.takarabio.com/documents/User%20Manual/NucleoSpin%20Virus%20RNA%20and%20DNA%20Purification%20User%20Manual/NucleoSpin%20Virus%20RNA%20and%20DNA%20Purification%20User%20Manual\\_Rev\\_02.pdf](https://www.takarabio.com/documents/User%20Manual/NucleoSpin%20Virus%20RNA%20and%20DNA%20Purification%20User%20Manual/NucleoSpin%20Virus%20RNA%20and%20DNA%20Purification%20User%20Manual_Rev_02.pdf)

Martinez-Gutierrez, M. and Ruiz-Saenz, J. (2016). Diversity of susceptible hosts in canine distemper virus infection: a systematic review and data synthesis. *BMC Vet Res.* 12:78.

Meerburg, B., Singleton, G. R., & Kijlstra, A. (2009). Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology*, 35(3), 221–270.

Meridian Life Science Inc, USA. MyTaq™ Red Mix.

[https://www.bioline.com/mwdownloads/download/link/id/2697/mytaq\\_red\\_mix\\_product\\_manual.pdf](https://www.bioline.com/mwdownloads/download/link/id/2697/mytaq_red_mix_product_manual.pdf)

Meridian Life Science Inc, USA. SensiFAST™ cDNA Synthesis Kit.

[https://www.bioline.com/mwdownloads/download/link/id/2788/p/i/pi-50476\\_sensifast\\_cdna\\_synthesis\\_kit\\_v9.pdf](https://www.bioline.com/mwdownloads/download/link/id/2788/p/i/pi-50476_sensifast_cdna_synthesis_kit_v9.pdf)

Murphy, F. A., Gibbs, E. P. J., Horzinek, M. C. and Studdert, M. J. (1999). San Diego, Calif. Academic Press. *Vet. Virol.*

Noyce, R. S., Delpeut, S. and Richardson, C.D. (2013). Dog nectin-4 is an epithelial cell receptor for canine distemper virus that facilitates virus entry and syncytia formation. *Virology*, 436(1): 210-220.

Shin, Y., Mori, T., Okita, M., Gemma, T., Kai, C., and Mikami, T. (1995). Detection of canine distemper virus nucleocapsid protein gene in canine peripheral blood mononuclear cells by RT-PCR. *The Journal of veterinary medical science*, 57(3), 439-445.

Sykes, J. E. (2014). Canine Distemper Virus Infection. Chapter 15 in: *Canine and Feline Infectious Diseases*. J. E. Sykes. Saint Louis, W.B. Saunders: 152-165.

Techangamsuwan, S., Banlunara, W., Radtanakantikanon, A., Sommanustweechai, A., Siriaroonrat, B., Lombardini, E. D. and Rungsipipat, A. (2015). Pathologic and Molecular Virologic Characterization of a Canine Distemper Outbreak in Farmed Civets. *Veterinary Pathology*. 52(4):724-731.

Tipold, A., Vandeveld, M. and Jaggy, A. (1992). Neurological manifestation of canine distemper virus infection. *Journal of Small Animal Practice*, 33, 466–470.

von Messling, V., Zimmer, G., Herrler, G., Haas, L. and Cattaneo, R. (2001) The hemagglutinin of canine distemper virus determines tropism and cytopathogenicity. *J Virol.*;75:6418–6427.

Wang, M., Yan, M., Xu, H., Liang, W., Kan, B., Zheng, B. and Xu, J. (2005). SARS-CoV Infection in a Restaurant from Palm Civet. *Emerging Infectious Diseases*, 11(12), 1860-1865. <https://doi.org/10.3201/eid1112.041293>.

Williams, E. (2001): Canine Distemper. In *Infectious Diseases of Wild Mammals* (Williams and Barker, (Eds.). Iowa State University Press, Ames, IO. 50-59.

## 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/>);

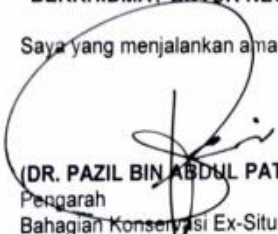
- c. Duplikasi semua sampel hidupan liar yang diambil perlu diserahkan melalui rakan saing Jabatan;
- d. Sebarang sampel tidak boleh dibawa ke luar negara;
- e. Berkongsi hasil penyelidikan seperti laporan/penerbitan kertas saintifik/tesis dengan Jabatan melalui rakan saing;
- f. Mengemukakan satu laporan 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)**  
Pegarah  
Bahagian Konservasi Ex-Situ  
b.p Ketua Pegarah  
Jabatan Perlindungan Hidupan Liar dan  
Taman Negara (PERHILITAN)

s.k.

Ketua Pegarah  
Timbalan Ketua Pegarah (Konservasi)  
Pegarah Bahagian Kawasan Perlindungan  
Pegarah PERHILITAN Terengganu  
Pegarah PERHILITAN Johor  
Penguasa Taman Negara Pahang  
Pegarah 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