Canine Distemper Virus Spillover Into Wildlife Population in Kampung Besul Lama, Terengganu Malaysia

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Article

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Abstract

Human wildlife gap has shrunk due to urbanization and agricultural expansion. Domestic animals and wild mammals are pushed closer together, enabling disease transmission between the two groups. Recent discovery in 2019 of *Awang Besul*, the first Malayan tiger to succumb to the canine distemper virus (CDV) in Kampung Besul Lama, Terengganu highlights the urgency of this issue. Thus, this study identified potential CDV hosts and antigen status of these to formulate efforts to prevent wildlife transmission in Kampung Besul Lama. Potential CDV hosts were captured using baited wildlife traps, and species were identified and documented. Biological samples were also obtained, utilizing nasal and ocular swabs for the detection of CDV via RT-PCR. Multiple different species of small mammals were trapped and three tree shrews *Tupaia gais* were positive for the CDV antigen. The discovery of CDV antigen in this species, suggests disease maintenance in small mammal populations. Movement of small mammals harbouring disease between Kampung Besul Lama and forests reserve could become the bridge of animal disease transmission between wildlife and domestic groups, which in turn could infect Malayan tigers. Introduction of the CDV virus to wild tiger populations via small mammals could lead to an extinction level event, thus understanding the mode of transmission of the CDV would benefit conservation effort here in Malaysia.

1. INTRODUCTION

The population and home-range of Malayan tigers (*Panthera tigris malayensis*) has declined steadily to less than 150 individuals in the latest National Tiger Survey (WWF Malaysia, 2022). In fact, decline in population has been attributed to habitat loss due to deforestation and plantation, poaching for the illegal trade of tiger parts, killings in area of human-tiger conflict as well as hunting of tiger's natural prey population (Shevade *et al.*, 2017). Urbanization and agricultural expansions have decimated forests in Malaysia with an annual estimate of 0.43Mha/y, leading to habitat fragmentation, constriction of species movement and human encroachment into wild territories (Giree *et al.*, 2013, Danneck *et al.*, 2023). Consequently, the diminished human-wildlife gap has enabled further challenges to conservation in the form of novel disease transmission to wildlife species.

Domestic animal diseases such as canine distemper (Ten *et al.*, 2021), feline parvovirus (Goodrich *et al.*, 2012), and feline immunodeficiency virus (Gilbert *et al.*, 2023), has been reported in wild tigers, suggestive of disease transmission from domestic groups to wild species. Predation of domestic animals seems to be the likely causative factor in disease transmission between these two groups (Gilbert, 2023). However, the potentiality of smaller mammals such as civets and tree shrews harbouring and transferring diseases must be acknowledged, considering their movement in and out of human settlements in search of food. Additionally, smaller mammals exist in much larger populations, enabling viruses such as the canine distemper virus to be propagated and maintained in an enzootic state (Wilkes, 2022).

Canine distemper virus of the genus Morbilivirus and family Paramyxoviridae is a single-strand RNA virus infecting carnivorous mammals and causes the highly contagious and fatal canine distemper infection (Deem *et al*., 2000; Beineke *et al*., 2015). Canine distemper virus has been reported to infect a wide range of hosts such as dogs, ferrets, wild dogs, foxes, jackals, coyotes, hyenas, cheetahs, seals, sea lions' dolphins and big cats such as lions, leopards and tigers (Carvalho *et al., 2012*). Transmission of the virus to a wide range of hosts includes via direct contact, oral, ocular and respiratory discharges as well as virus containing exudates (Loots *et al*., 2017). Pathophysiological effects of the canine distemper virus involve multiple organ failure, respiratory and gastrointestinal signs, secondary bacterial infection, neurological symptoms and severe transitory immunosuppression (Carvalho *et al., 2012*).

In Malaysia, a case of canine distemper virus has been reported in the case on *Awang Besul*, in Kampung Besul Lama, Terengganu. This headline case attracted national controversy due to a timid-looking tiger, roaming around the village area, displaying zero signs of aggression. Although *Awang Besul* is the first reported case of CDV in Malaysia, this novelty may indicate a more widespread threat to the critically endangered Malayan tiger. Thus, this study identified potential intermediate hosts in the first confirmed case of CDV in wild Malayan tiger. This study also conducted antigen detection of CDV in small mammals captured, as well as the techniques used to sample the different captured species. In addition, this study theorizes the relationship between smaller mammals towards the transmission of CDV to wild Malayan tigers.

2. MATERIALS AND METHODS

2.1 Location of study

The study was conducted from 19th July 2023 to 10th May 2024 at the Kampung Besul Lama, Terengganu. This area was chosen as the first location of study due to confirmed canine distemper virus in a Malayan tiger. Figure 1 shows the location of Kampung Besul Lama which is connected with the forested area, orchards and village settlement. The orchards areas at Kampung Besul Lama are chosen as study site due to high changes of a mixture of domestic species as well as wild small mammals' presence. The permit of wildlife research at the Kampung Besul Lama was approved by the Department of Wildlife National Parks (PERHILITAN) Peninsular Malaysia Reference: 100 – 34/1.24 Jld20(11).

2.2 Wildlife trapping and sampling using baited traps

Wildlife traps of two sizes were used for trapping of small mammals. First set of traps (n = 10; 18cm x 29cm x13cm) aimed towards smaller mammals such squirrels or rats. The second set of traps (n = 10; 40cm x 30cm x 65cm) aimed towards larger mammals such as civets, wild cats and other possible mammals. These wildlife traps were placed in peri-forested and orchard areas as well in areas of movement where *Awang Besul* was detected. Traps aimed at smaller mammals were placed on trees, nearby food sources (e.g fruit trees), and on the ground in bushes. Traps aimed larger mammals were placed in bushes, along wildlife pathways and around village areas where chicken coop was present. All traps were baited with bananas, jackfruit and palm oil kernel. The traps were checked twice daily (morning and evening) and the baits were replaced if damaged or spoilt. Figure 2 shows location of the areas in which traps are placed from wildlife sampling (grey circle) and movement of *Awang Besul* (red line).

Captured animals were anaesthetised using ketamine hydrochloride and xylazine according to the recommended doses based on the species (Thirumurugan *et al.*, 2004, Umami, 2002 and Pye, 2001) and recommendation by practicing wildlife veterinarians. Nasal and oral samples were collected using sterile swabs and preserved in virus transport medium for the detection of the CDV via RT-PCR. Anaesthetised mammals were placed in a dark area for recovery and released after 24 hours into the same site as capture. All animals' handlings were conducted according to the principles of laboratory animals care and use committee (Institutional Animal Care and Use Committee) approved by the Universiti Putra Malaysia (UPM/IACUC/AUP-R092/2017). The study was reported in accordance with ARRIVE guidelines (Percie du Sert *et al.*, 2020).

2.3 RT-PCR

Nasal and ocular swabs were stored in virus transport medium until it can be brought to the lab. The RNA extractions were done using physical methods as suggested by the test kit protocol. The RNA was converted to cDNA using the *Sensifast* cDNA synthesis kit. The cDNA was used for PCR analysis using the *MyTaq* Redmix PCR kit and gel electrophoresis were used to visualize the qualitative of CDV band.

2.4 Data analysis

Data obtained from the studies were analysed with MedCalc Statistical Software version 19.0.4. All data are reported as mean ± SD and statistical significance was described as a *P*-value of less than 0.05.

3. RESULTS AND DISCUSSION

Table 1 shows the species of small mammals were captured and recorded. Sampling yielded a total of 77 small mammals; *Tupaia gais* (n = 16), *Callosciurus notatus* (n = 9), *Callosciurus caniceps* (n = 7), *Sundasciurus tenuis* (n = 9), *Paradoxurus hemaphroditus* (n = 8), *Vivera tangalunga* (n = 1), *Leopoldamys sabanus* (n = 1), and *Rattus rattus* (n = 26). These small mammals species are believed to be the potential causative agents for the spillover of the canine distemper virus from domestic areas to wildlife habitats. Besides their ability to be infected by the virus, these small mammals exist in large numbers, enabling the maintenance of the canine distemper virus within their population in an enzootic state. Social interactions are common between species such as the tree shrews, which facilitates virus transmission and maintenance. Previous studies on the canine distemper virus shows that the potential hosts for the virus are limitless, with the virus being detected dogs, ferrets, wild dogs, foxes, jackals, coyotes, hyenas, cheetahs, seals, sea lions' dolphins and big cats such as lions, leopards and tigers (Carvalho et al., 2012).

Table 1
The small mammals captured and the antigen positive individuals detected

Species	**Number of individuals (n)**	**Relative abundance (n/Nx100)**	**CDV antigen positive**
Common tree shrew *Tupaia gais*	16	20	3
Plantain Squirrel *Callosciurus notatus*	9	11	0
Grey – Bellied Squirrel *Callosciurus caniceps*	7	9	0
Slender squirrel *Sundasciurus tenius*	9	11	0
Asian Palm Civet *Paradoxurus hemaphroditus*	8	10	0
Malayan Civet *Vivera tangalunga*	1	1	0
Long-tailed Giant Rat *Leopoldamys sabanus*	1	1	0
Field rat *Rattus rattus*	26	33	0
Total	**77**		3

Figure 3 shows the qualitative identification via conventional RT-PCR depicting antigen positive results for tree shrew (*Tupala glis*) samples from Kampung Besul Lama based on selected region in the H gene. Three tree shrews were detected to be antigen positive at time of sampling. This indicates that the tree shrews are infected by the CDV, thus have a high potential of spread to naive small mammals due to their large population size. Viral maintenance in reservoir populations has been demonstrated in other populations, such as the harp seals in the Northwest Atlantic (Puryear *et al.*, 2021). Although the canine distemper virus has been identified in multiple species worldwide, this is the first detection of the CDV antigen in tree shrews in Malaysia suggesting further research into viral carrying capabilities of this species. We hypothesise that these positive host could be asymptomatic or at the recovery phase of the viral infection, thus not displaying symptoms, but actively discharging virus. The positive result obtained, could indicate that the canine distemper virus is being maintained in small mammal populations, enabling viral survivability and infectibility to naive species when opportunity arises. Maintenance of CDV in small mammal population has been demonstrated by a study in Germany and USA where racoons were responsible for the spread of disease to domestic and wild species, with virus being maintained in the racoon population (Batista Linhares *et al.*, 2021, Lednicky *et al.*, 2004).

Results of PCR in qualitative analysis could indicate that small mammal is the reservoir host for the CDV transmission and are responsible for the infection of wild mammals. Despite other captured mammals testing negative, it does not indicate that they have never been exposed or recovered from a CDV infection. Previous study reported reduced sensitivity and specificity with RT-PCR with dissatisfactory detections during the early and end stage of the infection (Kim *et al.*, 2001). In addition, we theorize that these small mammals will move between human settlements and forested areas to forage and coincidentally transmit disease. Mapping the movement of small mammals would enable us to predict the possibility of disease transference. Due to the connectivity of some forests in Malaysia, maintenance of the CDV in small mammal populations would prove devastating to endangered species. Kampung Besul Lama at Terengganu is the first case of reported CDV in wild Malayan tiger. The same forest that hosts *Awang Besul* in Terengganu is connected to National Park, Pahang, Malaysia. If the transmission of CDV ever reaches the National Park, the miniscule tiger population left will be left at severe risk of extinction.

The theory of transmission of the CDV to *Awang Besul* is depicted in Fig. 4, with certain areas of potential interaction highlighted. Considering the original hosts of the CDV are dogs, the landscape of Kampung Besul Lama provides ample interactions between dogs and small mammals in the form of fruit orchards bordering peri forested areas, palm oil plantations and tropical forests. Sharing of the same environment between roaming dogs and small mammals, creates an optimal environment for virus transmission, suggesting the transmission of CDV to tree shrews enabling the potential maintenance of the virus in the population. Regarding *Awang Besul*, the fragmented forests surrounding the village areas may facilitate tiger movement through villages in search for food, and other socioecological interactions. This could lead to possible predation of infected dogs, or movement into regions inhabited by infected small mammals and vice versa, thereby increasing risk of infection and death of tigers. According to Gilbert *et al*., 2023, pathogen emergence in wildlife may be attributed to modified habitats with reduced biodiversity, altered climates and fragmented landscaped. This is seen in Kampung Besul Lama, where indiscriminate logging has led to fragmented forests causing wildlife movement thorough human settlements. Besides that, the African swine fever outbreak in wild boars in Malaysia has concurrently reduced prey population, predator movement to areas of human settlements in search of food (Luskin *et al*., 2023). Thus, these findings provide fundamental knowledge towards the hypothesis described above regarding the transmission of the canine distemper virus towards *Awang Besul*.

4. CONCLUSION

In conclusion, this study demonstrates the that CDV has infected small mammal populations in Kampung Besul Lama, Terengganu. The study documented different species located in peri forested areas and provided an insight towards the transmission of CDV to wild mammals. The knowledge obtained from this study will be used for further enhancement for wildlife conservation and enable more detail towards the study of ecology and biology.

Additionally, this work will provide solid foundations for the field of one health disease transmission in wildlife. Decades of research has documented wildlife disease transmission towards humans (Kruse *et al.*, 2004), but in this study, we highlighted the transmission of domestic animal disease towards wildlife. We hope that the knowledge gained from this study will further bridge the gap between humans and wildlife.

As mentioned, the forests connectivity could prove a point of transmission if the CDV is being maintained in small mammal populations. Thus, we recommend that domestic dogs and small mammals in human settlements on the edge of forests reserved are screened for CDV especially in areas of high risk and value such as villages in close proximity to primary forests that contain Malayan tigers.

Declarations

Author Contribution

B.A.L and H.A wrote the main manuscipt text. B.A.L and H.A prepared the figures and tables. F.M.K, K.K.H, R.A.W, M.A.K, M.L.A and T.R.P.T.A were involved in the sample collection process. B.A.L and M.F.M.S were involved in sample preparation, sample analysis and data recording. All authors reviewed the manuscript.

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Data Availability

The location and animal sampled are available and included in this study. Some of the confidential data are restricted to protect the endangered wildlife in the area of study. Data are available from corresponding author, Associate Professor Dr. Hafandi Ahmad (hafandi@upm.edu.my) for researchers who meet the criteria for access to confidential data.

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Figures

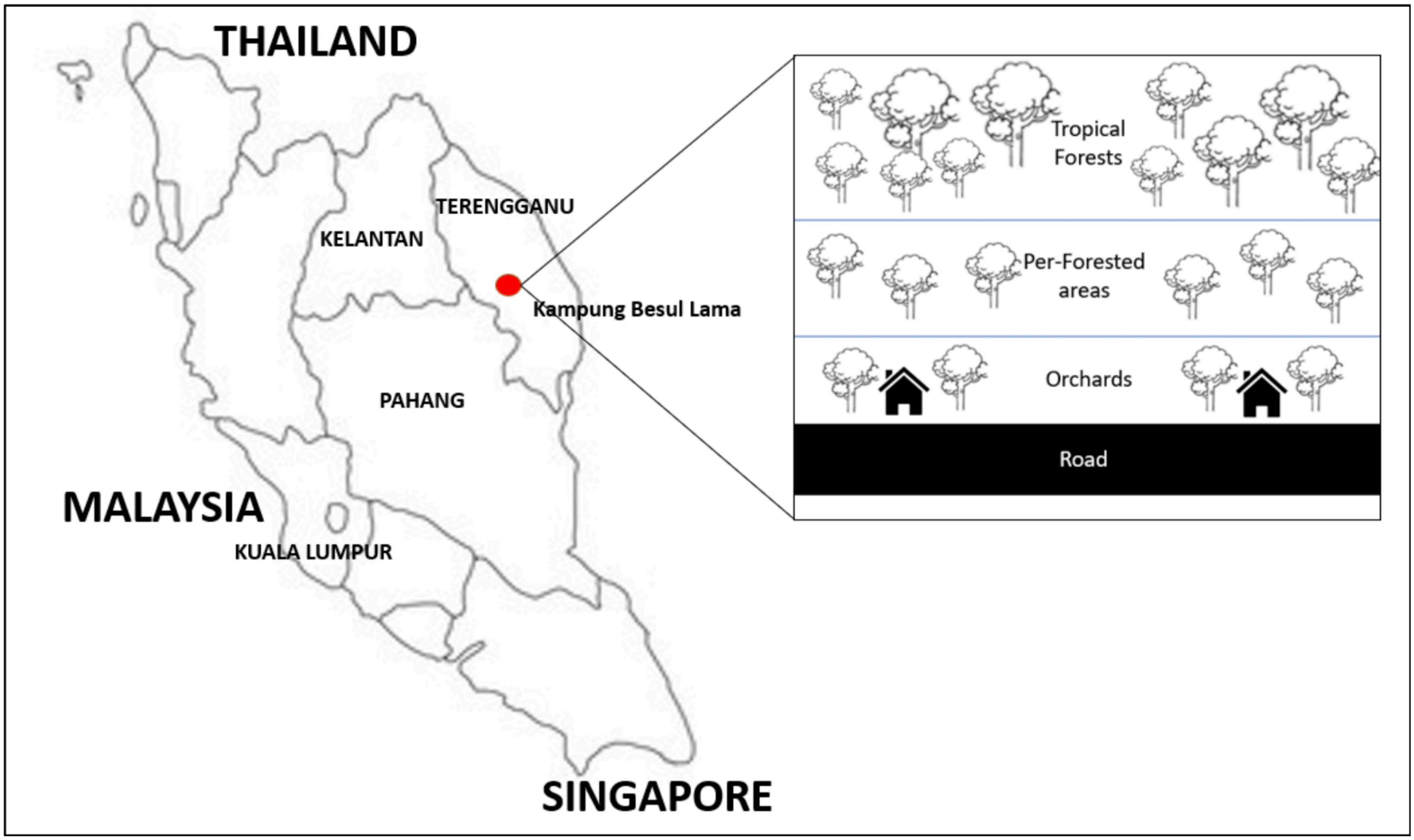

Figure 1

Location of Kampung Besul Lama in a map of Malaysia

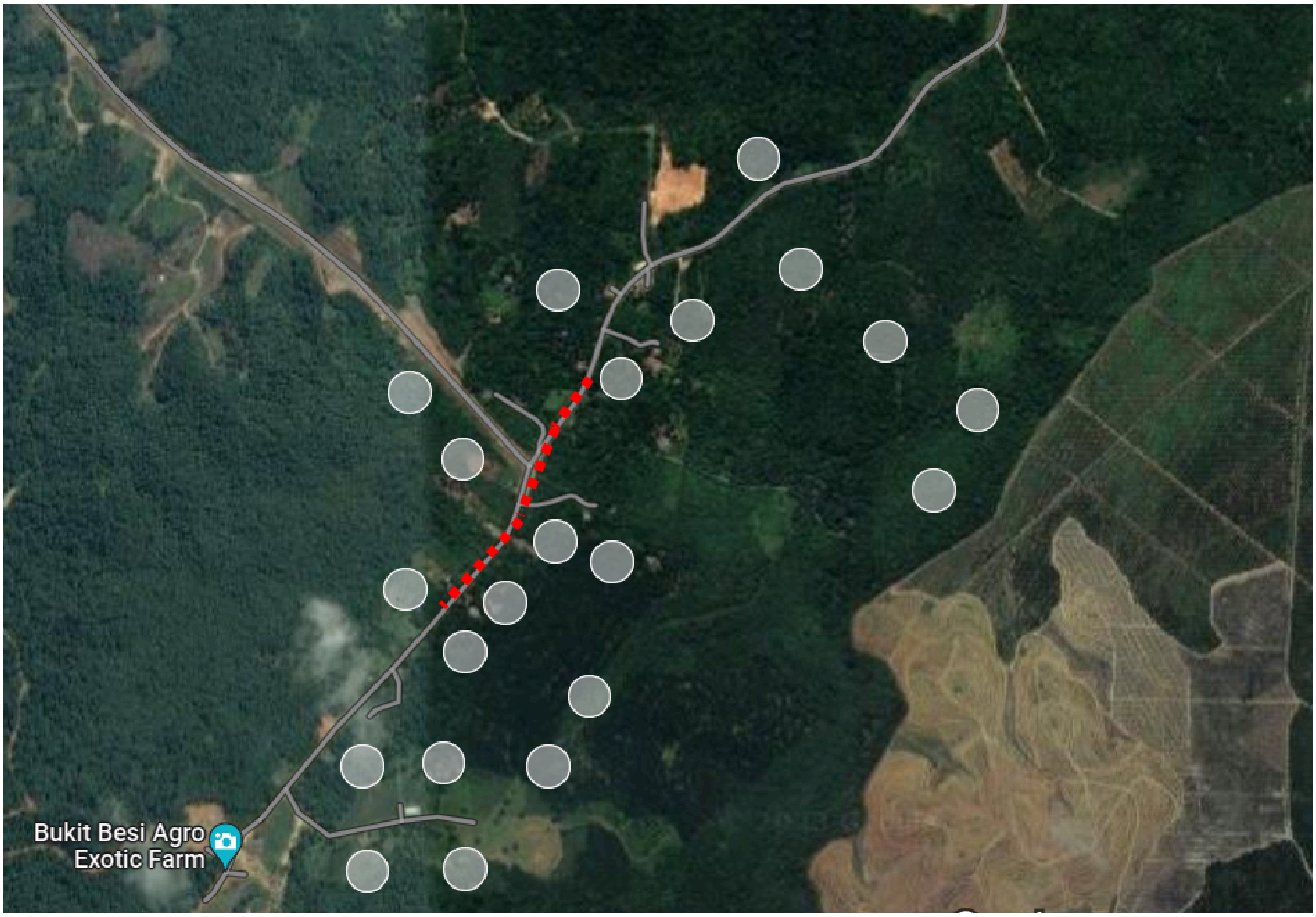

Figure 2

Location of the areas in which traps are placed from wildlife sampling (grey circle). Movement of *Awang Besul* is displayed on map (red line).

(https://earth.google.com/earth/d/1A_VYXtrya9lqPdqdaLWfghuhmgeT7qN?usp=sharin)

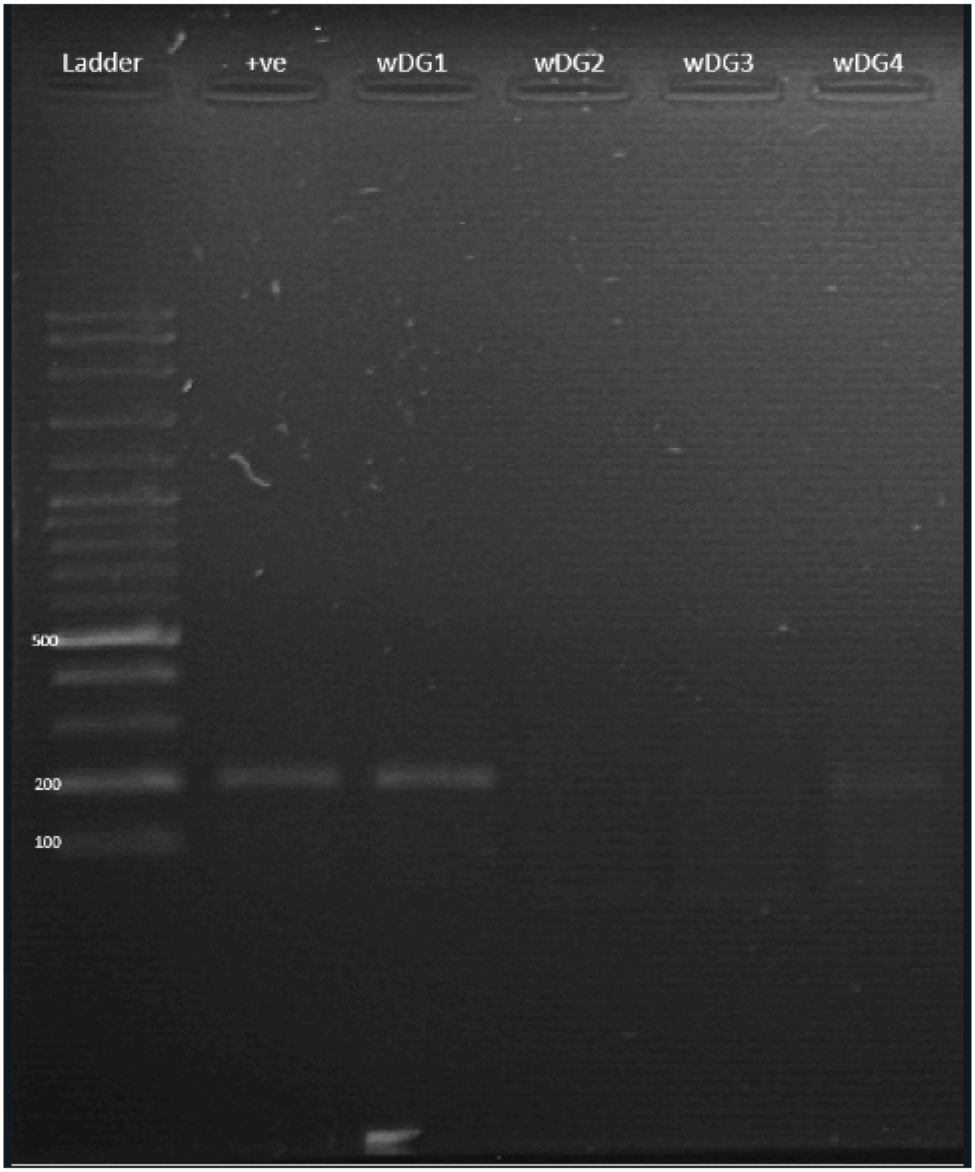

Figure 3

Qualitative identification via conventional RT-PCR depicting antigen positive results for Tree shrew *Tupala glis* (wDG1 and wDG4) samples from Kampung Besul Lama, Terengganu based on selected region in the H gene.

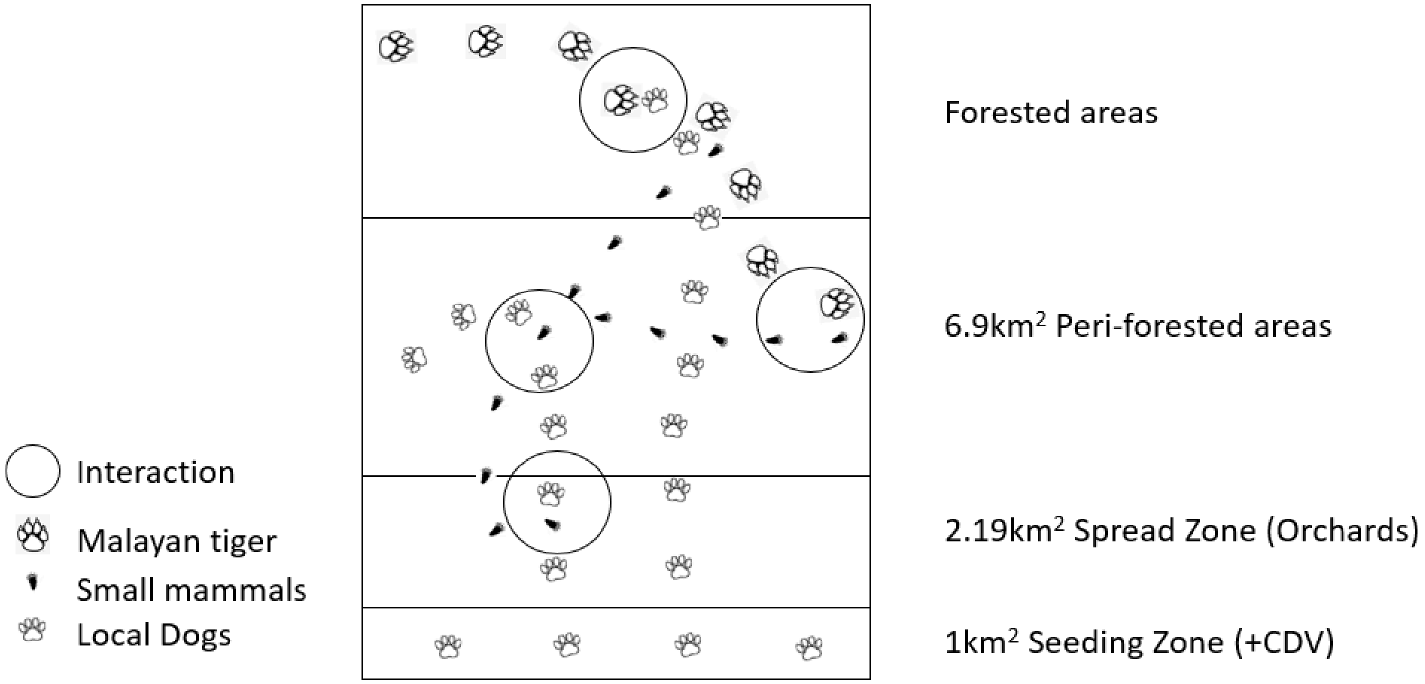

Figure 4

Hypothesis on interaction and transmission theory of CDV between village dogs, small mammals and Malayan tiger. Distance written was calculated according to Google Earth.