Thesis progression

Thesis title: Diversity of Microbial Pathogens and Cross-Species Transmission Potential of Candidate Virus in Rodents in Chanthaburi and Chiang Rai, Thailand

Thesis progression title: Diversity of Bacterial Profile in Rodents in Chanthaburi, Thailand

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1. Introduction and rationale

During the last decades, the majority of emerging infectious diseases (71.8%) caused by zoonotic pathogens originate from wildlife species (Jones, K.E. et al., 2008). Spillover of various zoonotic viruses from wildlife hosts to humans causes high-impact morbidity and mortality globally, including pandemics of severe acute respiratory syndrome related to zoonotic-origin *coronaviruses*, *lyssaviruses,* hemorrhagic fevers caused by *hantaviruses*, *flaviviruses, poxviruses, hepatitis E virus,* and influenza A virus (Camp et al., 2022). The emergence of these diseases occurs when an animal virus switches host to humans and is transmitted within human populations. Anthropogenic changes, including increasing human population density, increasing international travel, land-use change, and urban sprawl, appear to be drivers of the spillover and spreading of zoonotic viruses to humans. Many studies have revealed that land use change caused increased interaction between wildlife, domestic animals, and humans (Patz JA et al., 2004). Since types of interactions are favorable to cross-species transmission, the emerging disease is automatically promoted and a zoonotic risk to human health is scarce.

Rodents (Order *Rodentia*) are one of the largest and most diversified groups of mammals comprising over 2,200 species from five suborders including *Anomaluromorpha, Castorimorpha, Hystricomorpha, Myomorpha,* and *Sciuromorpha*. Some of the most common families are *Castoridae* (e.g., beavers), *Cricetidae* (e.g., hamsters, voles, new-world rats, and mice), *Muridae* (e.g., brush-furred mice, sand rats, old-world rats, and mice), and *Sciuridae* (e.g., squirrels). The *Muridae* is the largest family, containing approximately 1,383 species (Naluepanat, 2022).

Rodents are recognized as an important disease reservoir, with potential health impact globally for diverse zoonotic diseases. These include plague (Walker DH et al., 1996),

hemorrhagic fever with renal syndrome (HFRS) (Watson DC et al., 2014), scrub typhus (Lerdthusnee K et al., 2008), Lassa fever (Meerburg BG et al., 2009), and leptospirosis (Suwannarong & Chapman, 2015). Rodents-borne diseases can be transmitted to humans by 2 main routes including 1) direct transmission by biting, or inhaling (direct contact), and 2) contamination of food and water with urine or feces of rodents, and through arthropod vectors (indirect contact). Various types of pathogens transmitted by rodents include bacteria, viruses, protozoan parasites, and nematodes, such as *Leptospira* spp., *Salmonella* spp., coronaviruses, hantaviruses, Leishmania, *Toxoplasma gondii,* and *Trichinella* spp.

Rodents inhabit all regions of the earth except Antarctica and some islands (Akhtar et al., 2023). Cities are unique ecosystems where dense human populations and their animals live in relative proximity to wildlife species (Camp et al., 2022). Urbanization is rapidly modifying most of Southeast Asia, altering the structure and function of the landscape as well as the frequency and intensity of interactions between humans, animals, and the environment. While rodent species diversity has decreased with increasing urbanization, these changes have the potential to exacerbate many of the region's existing health concerns, including those posed by zoonoses. Urbanization affects the ecology of animal reservoirs, arthropod vectors, and pathogens, potentially increasing the risk of transmission of several zoonotic diseases in urban areas (Blasdell et al., 2022). Therefore, we conducted the specimen collection from rodents in 2 provinces of Thailand including Chanthaburi and Chiang Rai. These provinces reflect a diversity of biological, ecological, and societal contexts. Each includes a provincial city, district towns, sub-district towns, and a large number of villages, nature areas, contiguous forest areas, national parks, and scenic sites, which are often frequented by tourists and other visitors. Chanthaburi Province is a Thailand-Cambodia border and Chiang Rai Province is a Thailand-Myanmar- and Lao border. These areas also have large markets and are crowded with Thai and foreigners trading between the countries.

Limited studies on the diversity and prevalence of microbial pathogens and the cross-species transmission potential of novel viral strains are carried out in rodents in Thailand. For the detection of microbial pathogen diversity, PCR assays are a convenient standard method that depends on primers designed from known diversity and may lead to bias focusing only on interested microorganisms (Muzeniek et al., 2023). An increasing number of studies are using metagenomic techniques to record the wide range of disease diversity in animal species. Metagenomic next-generation sequencing (mNGS) is a powerful and widely significant tool

for discovering novel viruses in recent years that could be used to investigate diversity in ecological systems. Given the huge diversity of microbial pathogens, especially viruses, and the increasing frequency of direct and indirect interactions at the interface between humans and rodents, it is reasonable to expect that there are numerous unreported viruses and cross-species transmission events. Thus, this study aims to investigate the diversity and prevalence of microbial pathogens through 16S rRNA sequencing and mNGS and construct the phylogenetic tree of viral families identified in rodents for a selected candidate virus to determine the cross-species transmission potential in rodents in Chanthaburi and Chiang Rai, Thailand.

2. Hypothesis and objective

Hypothesis

1. The diversity and prevalence of bacterial pathogens in rodents from different habitats are different.
2. There is a candidate virus that has the potential of cross-species transmission circulating in rodents.

Objectives

1. To investigate the diversity and prevalence of bacterial pathogens in rodents from different habitats.
2. To determine the virome profile in rodents from different habitats.
3. To construct the phylogenetic tree of virus from rodents for the selection of candidate virus.
4. To determine the cross-species transmission potential of a candidate virus in rodents in Chanthaburi and Chiang Rai, Thailand.

3. Conceptual framework

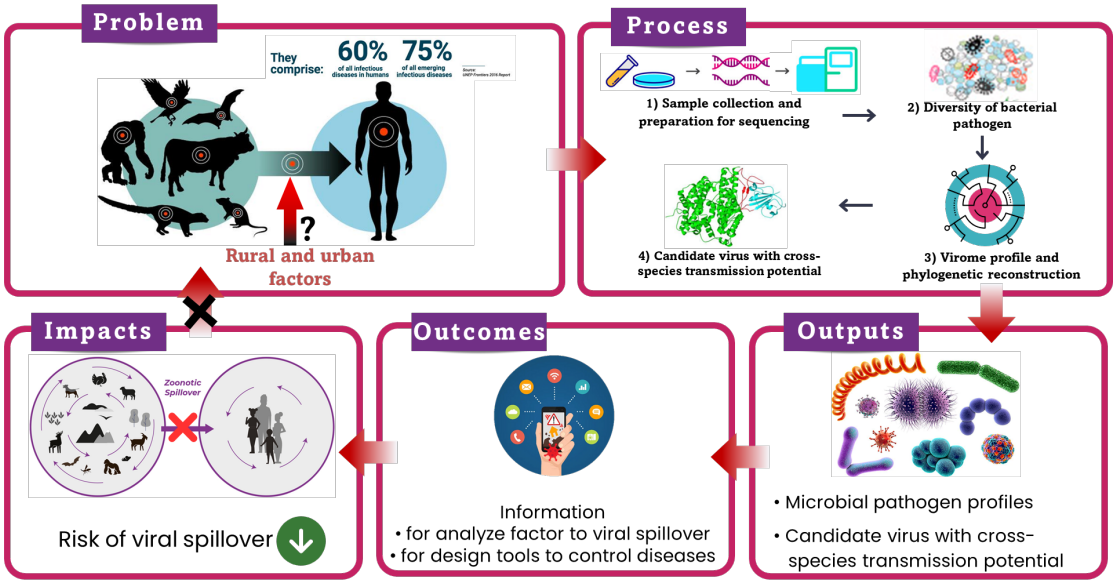

Figure 1 Conceptual framework

4. Study design

This study is divided into 4 parts (Figure 2) including:

Part 1 Sample collection and preparation for sequencing

Part 2 Investigation of the diversity of bacterial pathogens in rodents of each habitat

Part 3 Determination of virome profile in rodents of each habitat, and phylogenetic reconstruction

Part 4 Determination of candidate viral strain with cross-species transmission potential

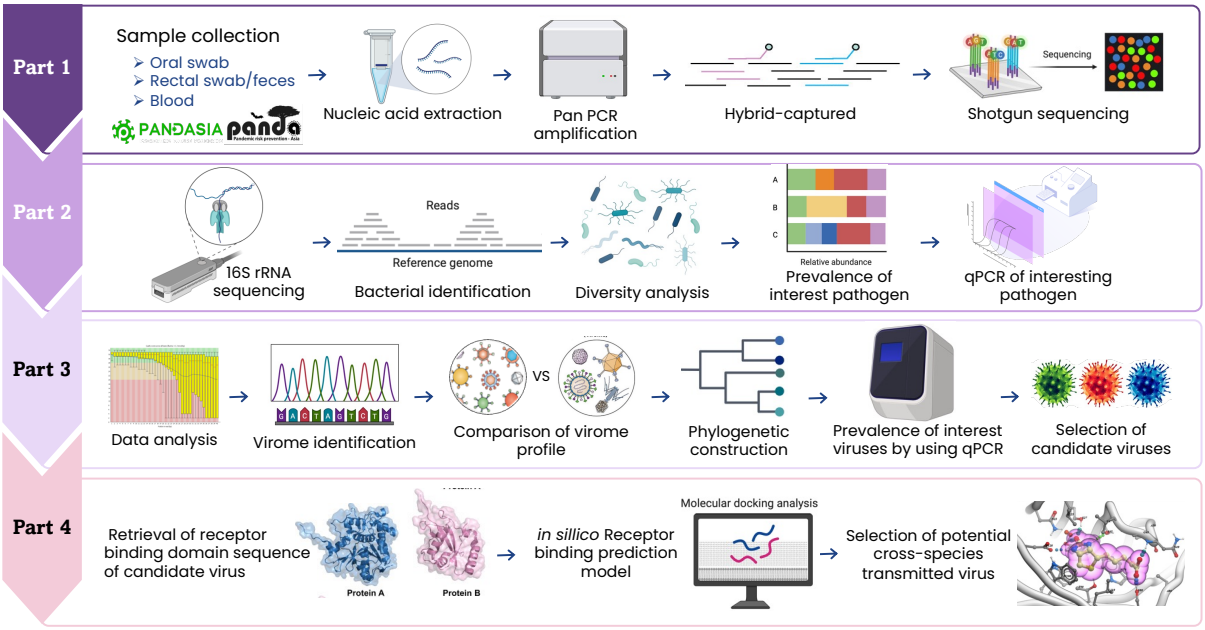

Figure 2 Study design

5. Materials and methods

5.1 Sample collection

A total sample of 320 rodents for the identification of virome profile was expected from 20 rodent samples x 4 locations x 2 seasons x 2 years. On the other hand, a total sample of 80 rodents for the identification of bacterial profile was expected from 20 rodent samples x 4 locations.

Selection of locations will be selected based on the following criteria including 1) preliminary information on wildlife contact and consumption in the areas; 2) preliminary information on wildlife presentations/habitats; 3) strong collaboration and coordination of local health facilities, residents, and authorities; and 4) results of the scoping visits.

We collected the rodent samples in Soi Dao district and Pong Nam Ron district in Chanthaburi province, and Wiang Kaen districts and Mae Fah Laung district in Chiang-Rai province. We will collect the samples in dry season and wet season between the years 2024-2025 and collect in 4 interphases, including 1) household; 2) forest; 3) dumpsite; and 4) orchard. Rodent samples from the PANDASIA and PANDA projects consist of oral swabs, rectal swabs or feces, and blood.

Rodents were trapped alive using 25 mesh wire traps (one per 20×20 m) baited with fresh corn in 100×100 m sampling plots in each study environment. Traps were set overnight (18:00-06:00) for 7 consecutive nights. Captured rodents were anesthetized in a chamber using isoflurane inhalation. Captured rats will be weighed, measured, and identified as species using morphological characteristics. Captured rodents will be implanted with a microchip for mark-release-recapture studies and population size estimation.

Oral and rectal swabs were placed in AVL buffer. Blood was collected in Capillary EDTA Micro Blood Collection Tube. After getting samples, swab samples were rotated using vortex for 15 seconds and the tip was pressed against the inner edge of the microtube with force to make sure we removed all liquid from the soft tip of the swab. The swab was placed in the biohazard waste bag provided. The microtube was closed the cap tightly and placed the tube into the cryobox. At the field office, store the samples at 4°C and ship them to Khon Kaen University (KKU) within 1 month. At KKU, freeze the samples at -20°C before nucleic acid extraction.

5.2 Nucleic acid extraction

Individual pool samples consist of 400 µL from oral swab, rectal swab, and blood transfer to the buffer AVL-carrier RNA in the microcentrifuge tube. The QIAamp Viral RNA

Mini Kit (Qiagen) was used for nucleic acid extraction according to the manufacturer's guidelines with slight modifications. The first elution step was divided into 2 tubes, with 35 µL in each tube for storage and hybrid- capture-based viral enrichment, and the 60 µL buffer AVE was added for the second elution, then divided into 2 tubes, with 30 µL in each tube to perform pan-PCR and nanopore sequencing. The extracted nucleic acid samples were stored at -80°C.

5.3 16S rRNA sequencing

To investigate the diversity of bacterial profiles in rodents from in different habitats in Chanthaburi and Chiang Rai, Thailand, the nanopore sequencing was performed. The nucleic acid from the 2nd elution was used as a template for 16S rRNA amplification by PCR. The PCR reaction was performed using the 27F forward primer (5′-AGAGTTTGATCCTGGCTCAG-3′) and the 1492R reverse primer (5′-TACGGYTACCTTGTTACGACTT 3′). PCR amplification of 16S rRNA genes was conducted using the 16S Barcoding Kit (Oxford Nanopore Technologies, Oxford, UK) containing the 27F/1492R primer set, whose PCR components for 16S amplification are PCR1 as shown in Table 1.

Table 1 PCR components for 16S amplification

PCR1	**10x**
KOD PCR maser mix	5 µL
16S F primer (27F) (10 µM)	0.3 µL
16S R primer (1492R) (10 µM)	0.3 µL
RNAase free water	3.4 µL
Total master mix	9 µL
DNA template	1 µL

Amplification was performed using an Applied Biosystems Veriti™ Thermal Cycler (Thermo Fischer Scientific, Waltham, MA, USA) with the following PCR conditions (Table 2).

Table 2 Thermal cycler Conditions

Thermal cycler condition			PCR1 primer cycle	PCR2 barcode cycle
PCR cycling step	**Temp**	**Time**		
Initial denaturation	98 °C	2 min	1	1
Denaturation	98 °C	10 sec	30	5
Annealing	60 °C	10 sec		
Extension	68 °C	10 sec		
Final extension	68 °C	5 min	1	1

PCR2 components for screening 16S amplification as shown in Table 3, which amplified with the following PCR conditions. Each PCR2 product for screening was subjected to electrophoresis on 1% agarose gel for 30 min at 100 V, and bands were checked for products of the expected size around 1,500 bp under UV light.

Table 3 PCR components for screening 16S amplification

PCR2	10x
KOD PCR maser mix	5 µL
Forward barcode	0.15 µL
Reverse barcode	0.15 µL
RNAase free water	3.7 µL
Total master mix	9 µL
PCR1 product	1 µL

PCR2 components for upscale 16S amplification as shown in Table 4, which amplified with the following PCR conditions. Barcode primer for upscale in strip PCR tubes that have been already mixed forward and reverse primer. One barcode mix per 1 sample is required.

Table 4 PCR components for upscale 16S amplification

PCR2	50x	20 reactions
PCR master mix	25 µl	500 µl
RNAase free water	18.5 µl	370 µl
Total master mix	43.5 µl	870 µl
Barcode Mix	1.5 µl	
PCR1 product	5 µl	

The library was prepared following the ONT protocol "Ligation sequencing DNA V14 (SQK-LSK114)" with a barcode's expansion pack. Primers used for amplification were 27F and 1492R, which amplifed the 16S rRNA gene spanning the variable regions V1–V9 and which were provided in the Rapid 16S amplicon barcoding kit. The final library was quantified using the Qubit™ dsDNA HS Assay Kit (Invitrogen™) and a Qubit 4.0 Fluorometer (Invitrogen™). The library was sequenced with the MinION using flow cells r.10.4.1 according to the manufacturer's instructions. These flow cells allow the sequencing of up to 96 samples per run.

For bacterial identification, the sequencing data were obtained, and basecalling was done using the GPU Guppy Basecalling Software with a fast base-calling algorithm. Approximate taxonomic identification of the sequences obtained from 16S rRNA sequencing was achieved through CZID (https://czid.org) to quickly identify bacterial sequences in metagenomic sequence datasets. The relative abundance (p_i) is the proportional representation of a species in a community or sample of a community. The relative abundance (p_i) of each species is expressed as the following formula (Achacoso, S. C., 2016).

$$P_i = \frac{n_i}{N} \text{ x } 100$$

where

n_i is the number of individuals of the same species

N is the total number of all species

6. Results

6.1 Sample collection

A total of 172 captured rodents in dry season in the year 2024 belong to 4 genera, and 8 species, of which *Rattus exulans* is a dominant species, followed by *Rattus rattus*. The captured rodents were mostly trapped in households, followed by the forest (Table 5).

Table 5 A total of captured rodent samples in dry season

	Household	Forest	Dumpsite	Orchard	Total
Berylmys berdmorei	3	NA	1	NA	**4**
Rattus rattus	26	12	7	7	**52**
Rattus exulans	88	1	1	NA	**90**
Rattus andamanensis	NA	12	NA	2	**14**
Rattus rattus/andamanensis	NA	1	NA	NA	**1**
Maxomys surifer	NA	3	NA	NA	**3**
Mus caroli	1	NA	NA	1	**2**
Mus cervicolor	NA	NA	4	NA	**4**
Mus pahari	NA	1	NA	NA	**1**
***Mus* spp.**	NA	NA	1	NA	**1**
Total	**118**	**30**	**14**	**10**	**172**

(NA: not available)

In Chanthaburi province, we collected a total of 70 rodent samples, belonging to 4 genera, and 6 species, of which *Rattus exulans* is the most common species, followed by *Rattus rattus*. *Berylmys berdmorei* and *Mus* spp. are the least common species, found in households and dumpsites, respectively. The captured rodents were mostly trapped in households, followed by the forest (Table 6).

Table 6 Number of rodent samples in Chanthaburi province

	Household	Forest	Dumpsite	Orchard	Total
Berylmys berdmorei	1	NA	NA	NA	**1**
Rattus rattus	13	8	3	5	**29**
Rattus exulans	29	1	NA	NA	**30**
Mus caroli	1	NA	NA	1	**2**
Maxomys surifer	NA	3	NA	NA	**3**
Mus cervicolor	NA	NA	4	NA	**4**
***Mus* spp.**	NA	NA	1	NA	**1**
Total	**44**	**12**	**8**	**6**	**70**

(NA: not available)

When considering each habitat, our data showed that *Rattus exulans* was a dominant species in the household, followed by *Rattus rattus*. *Rattus rattus* was mostly found in the forest and orchard, but *Mus cervicolor* is the most common species in the dumpsite and was only found in this habitat. As shown in Figure 3, the *Rattus rattus* can be captured from every habitat.

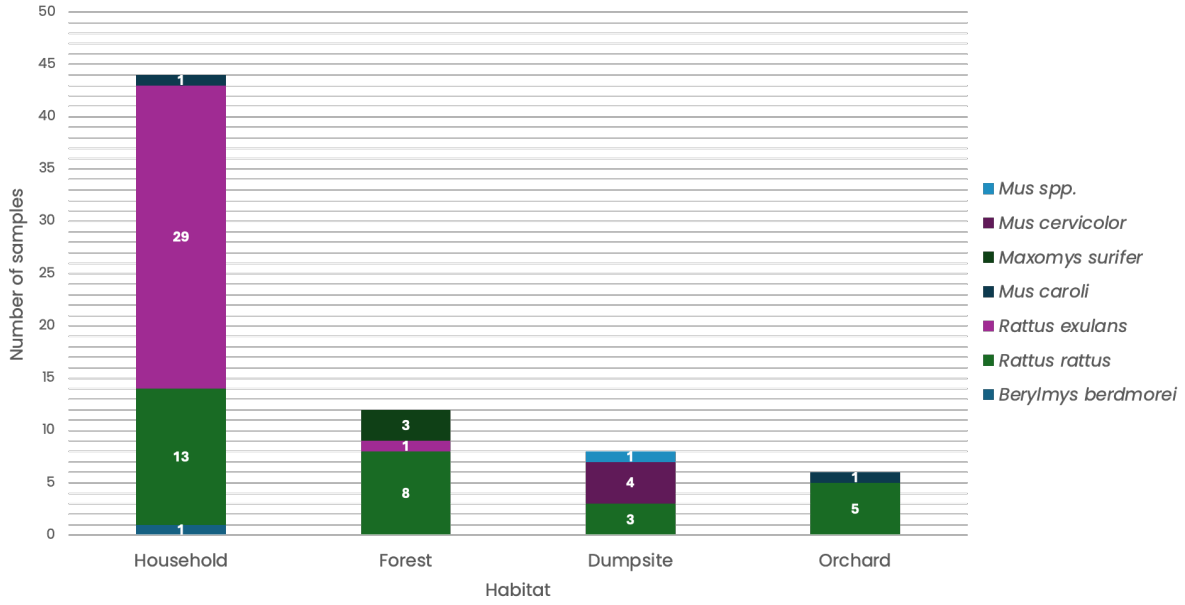

Figure 3 Number of rodent samples in Chanthaburi province

In Chiang-Rai province, we collected a total of 102 rodent samples, belonging to 3 genera, and 5 species, of which *Rattus exulans* is the most common species, followed by *Rattus rattus* as seen in Chanthaburi province. *Mus pahari* and *Rattus rattus/andamanensis* are the least common species. Both species were found in forests. The captured rodents were mostly trapped in households, followed by the forest (Table 7).

Table 7 Number of rodent samples in Chiang-Rai province

	Household	**Forest**	**Dumpsite**	**Orchard**	**Total**
Berylmys berdmorei	2	NA	1	NA	**3**
Rattus rattus	13	4	4	2	**23**
Rattus exulans	59	NA	1	NA	**60**
Rattus andamanensis	NA	12	NA	2	**14**
Mus pahari	NA	1	NA	NA	**1**
Rattus rattus/andamanensis	NA	1	NA	NA	**1**
Total	**74**	**18**	**6**	**4**	**102**

(NA: not available)

When considering each habitat, our data showed that *Rattus exulans* is a dominant species in the household, followed by *Rattus rattus*. *Rattus andamanensis* and *Rattus rattus* were mostly found in the forest and dumpsite, respectively. In the agricultural area, 2 species were found including *Ratus rattus* and *Rattus andamanensis.* As shown in Figure 4, *Rattus rattus* can be captured from every habitat as seen in Chanthaburi province.

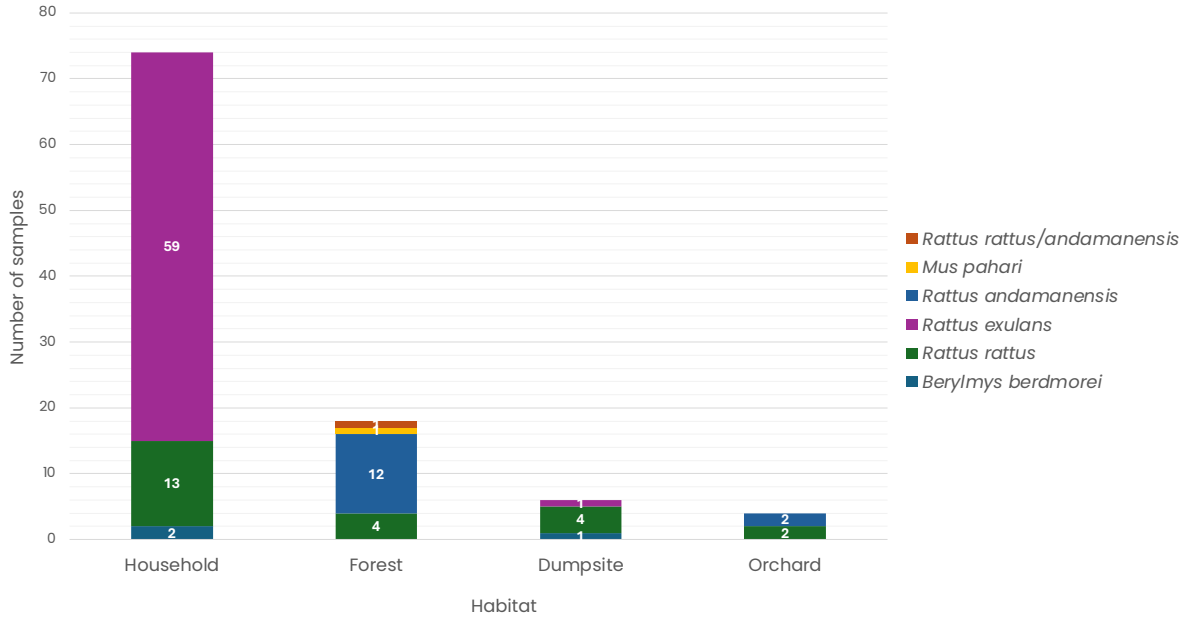

Figure 4 Number of rodent samples in Chiang-Rai province

6.2 Nucleic acid extraction

Currently, a total of 172 samples of nucleic acid extraction in the dry season in both Chanthaburi and Chiang Rai provinces have been completed. The final nucleic acid was quantified using the Qubit™ RNA BR Assay Kit (Invitrogen™) and a Qubit 1.0 Fluorometer (Invitrogen™) before further analysis. The assay kit is designed to be accurate for initial RNA sample concentrations of 0.5 to 1,200 ng/µL, providing a detection range of 10 to 1,200 ng.

6.3 16S rRNA sequencing

To investigate the diversity of bacterial profile in rodents in Chanthaburi, Thailand, the MinION Nanopore sequencing using flow cells r.10.4.1 was performed. The sequencing data were obtained, and basecalling was done using the GPU Guppy Basecalling Software with a fast base-calling algorithm. Approximate taxonomic identification of the sequences obtained from 16S rRNA sequencing was achieved through CZID (https://czid.org) to quickly identify bacterial sequences in metagenomic sequence datasets.

The raw data obtained from Nanopore sequencing found that the number of read counts per sample was in the range between 9,474 and 24,151 reads. Barcode06, which is the *Rattus rattus* found in households, has the highest number of reads at 24,151 reads, followed by Barcode02 *Maxomys surifer* from the forest with 19,943 reads. Barcode03, which is the *Rattus exulans* found in households, has the lowest number of reads at 9,474 reads (Figure 5).

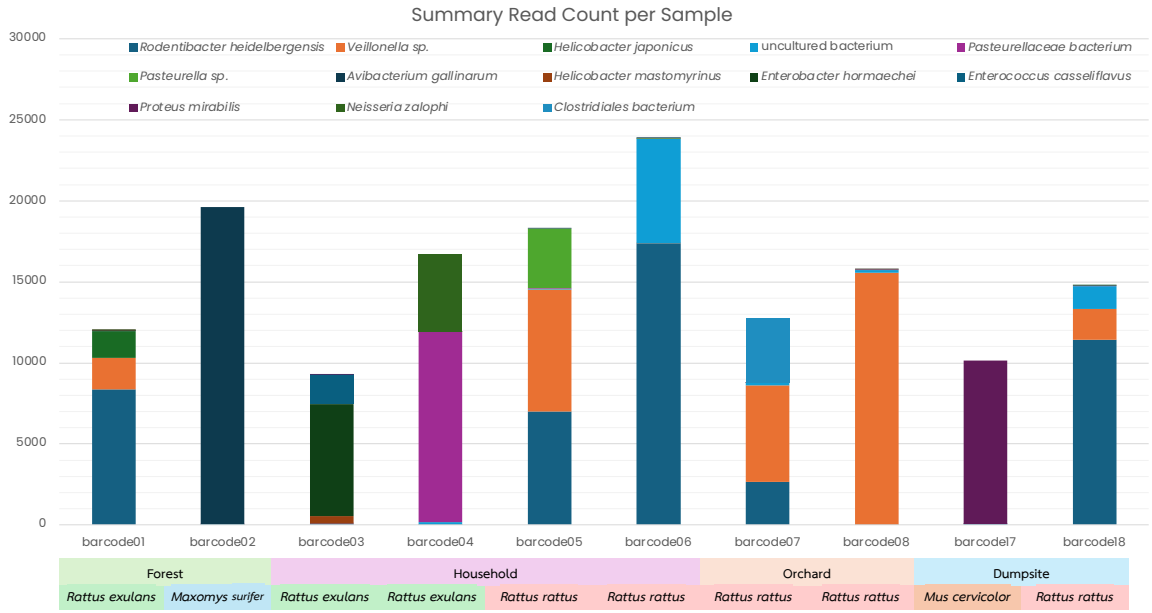

Figure 5 Summary read count per sample

To increase the accuracy of identifying bacterial species from sequencing or data analysis, we used 1% cut-off from the total number of reads to remove non-qualified bacterial species. If a bacterial species has a read count lower than the cut-off value, we do not analyzed that species to determine the relative abundance value and classify it as an unqualified read (Table 8).

Table 8 Summary Read Count per Sample

Habitat	Rodent species	Barcode	Total read	cut-off 1%
Forest	*Rattus exulans*	01	12242	122.42
	Maxomys surifer	02	19943	199.43
Household	*Rattus exulans*	03	9474	94.74
		04	16912	169.12
	Rattus rattus	05	18535	185.35
		06	24151	241.51
Orchard	*Rattus rattus*	07	12857	128.57
		08	15981	159.81
Dumpsite	*Mus cervicolor*	17	10411	104.11
	Rattus rattus	18	15022	150.22

Bacterial species with a read count greater than the cut-off value of 1% were calculated for the relative abundance. The relative abundance (p_i) is the proportional representation of a species in a sample of a community, which is calculated from the following formula: $P_i = \frac{n_i}{N}$ x 100 (Table 9).

Table 9 Relative Abundance of Bacterial profile in Chanthaburi province

Habitat	**Forest**		**Household**				**Orchard**		**Dumpsite**	
Rodent species	**R. *exulans***	**M. *surifer***	**R. *exulans***		**R. *rattus***		**R. *rattus***	**M. *cervicolor***	**R. *rattus***	
Barcode	01	02	03	04	05	06	07	08	17	18
Rodentibacter heidelbergensis	68.28	0.01	0.13	0.02	37.84	71.98	20.63	0.11	0.01	76.06
Veillonella sp. (*)	15.85	0.02	0.08	0.05	40.50	0.06	46.25	97.34	NA	12.56
Helicobacter japonicus	13.49	0.01	NA	NA	NA	NA	NA	NA	NA	NA
Helicobacter mastomyrinus	0.01	NA	4.80	0.01	NA	NA	NA	NA	NA	NA
Uncultured bacterium	0.29	0.18	0.35	1.09	0.33	26.62	1.31	1.04	0.48	9.55
Pasteurellaceae bacterium (*)	0.13	0.03	0.26	69.26	0.11	0.10	0.08	0.09	0.01	0.08
Pasteurella sp. (*)	0.06	NA	NA	0.02	19.89	0.02	0.02	0.03	NA	0..04
Avibacterium gallinarum	0.01	98.09	0.06	0.01	0.02	0.01	0.01	NA	0.02	0.02
Enterobacter hormaechei (*)	0.01	NA	72.82	NA	NA	NA	NA	NA	NA	NA
Enterococcus casseliflavus (*)	NA	NA	19.29	NA	NA	NA	NA	NA	NA	NA
Proteus mirabilis (*)	NA	NA	0.01	NA	NA	NA	0.01	0.01	96.86	NA
Neisseria zalophi	NA	NA	NA	28.39	NA	NA	NA	NA	NA	NA
Clostridiales bacterium (*)	NA	NA	NA	NA	NA	NA	30.79	NA	NA	NA
Unqualified read	1.88	1.67	2.18	1.14	1.32	1.20	0.89	1.38	2.62	1.70

(*) Bacterial pathogen

(NA: not available)

The result shown in the Table 9 and Figure 6 indicated that barcode 02, which is *Maxomys surifer* from the forest, and barcode 17, which is *Mus cervicolor* from the dumpsite, found only one species of bacteria that passed the cut-off value: *Avibacterium gallinarum* with a relative abundance (RA) value of 98.09%, and *Proteus mirabilis* with an RA value of 96.86%, respectively.

Rattus exulans from the forest (barcode 01), *Rattus rattus* from the household (barcode 06) and the dumpsite (barcode 18) were found to be *Rodentibacter heidelbergensis*, as the dominant bacterial species in the sample with RA values of 68.28%, 71.98%, and 76.06%, respectively.

Rattus exulans from the household were in a total of 2 pools, but the results from each pool showed different species of dominant bacteria. *Enterobacter hormaechei* was found in barcode 03 with RA value 72.82%, but *Pasteurellaceae* bacterium was found in barcode 04 with RA value 69.26%.

Rattus rattus from the household (barcode 05) and the orchard (barcode 07), and *Mus cervicolor* in the orchard (barcode 08) were found to have *Veillonella* sp. as a dominant bacterial species in the sample with RA values of 40.50%, 46.25%, and 97.34%, respectively.

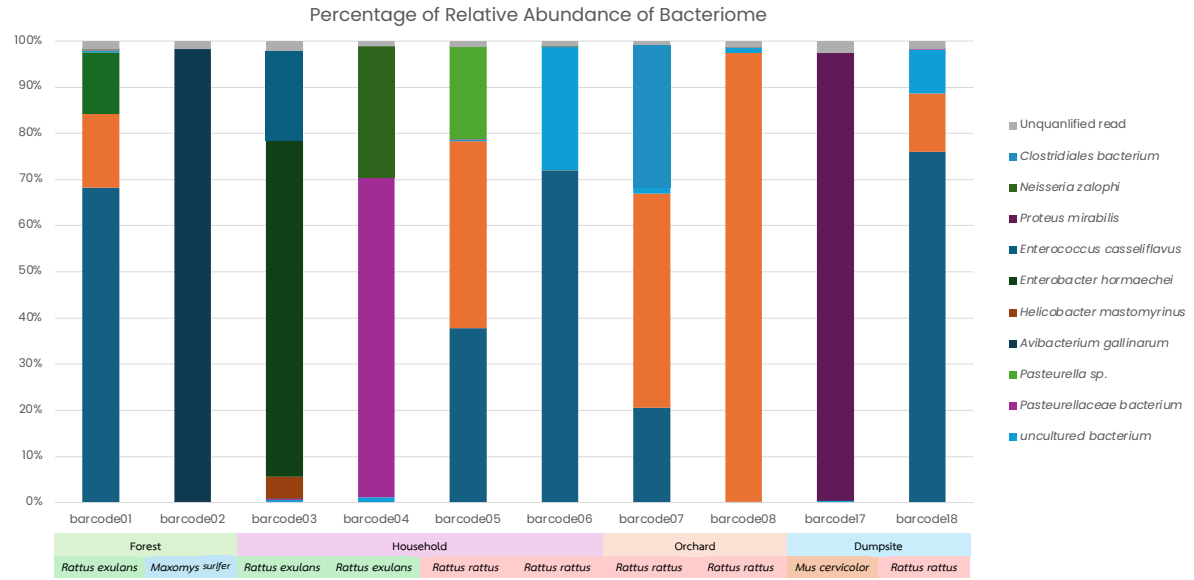

Figure 6 Relative Abundance of Bacterial profile in Chanthaburi province

7. Conclusion

A total of 172 captured rodents in dry season in the year 2024, belonging to 4 genera and 8 species. To decide on the selection of pool samples, we used nanopore sequencing to investigate the preliminary result of the similarity or difference of bacterial community between groups. We were sequencing by pool samples of Chanthaburi province, Thailand, according to rodent species and habitats. Five out of ten samples (*Rattus exulans* in forests (barcode01), *Mus cervicolor* in orchards (barcode08), and *Rattus rattus* in households (barcode05), orchards (barcode07), and dumpsites (barcode18)) include the most prevalent bacteria pathogen, which is *Veillonella* sp. Moreover, *Pasteurellaceae* bacterium, *Pasteurella* sp., *Enterobacter hormaechei*, *Enterococcus casseliflavus, Proteus mirabilis*, and *Clostridiales* bacterium represent bacterial pathogens identified in R. *exulans* from household (barcode04), R. *rattus* from household (barcode05), R. *exulans* from household (barcode03), R. *rattus* from dumpsite (barcode17), and R. *rattus* from orchard (barcode18), respectively.

The results showed that the different rodent species resided in the same habitat and rodents of the same species but residents in different habitats, they have a different bacterial community. Even rodents of the same species that live in the same interphase, they also have a different bacteria diversity.

8. Thesis Plan

Table 10 Thesis Plan

Activities	Timelines																	
	2023		2024				2025				2026				2027			
	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1. Coursework																		
2. Literature review and planning																		
3. Qualify examination																		
4. Proposal examination																		
Part 1 Sample collection and preparation for sequencing																		
5. Sample collection																		
6. Nucleic acid extraction																		
7. Pan PCR amplification																		
8. Hybrid-capture based viral enrichment																		
9. Shotgun NGS																		

Part 2 Diversity of bacterial pathogen in rodents of each habitat																		
10. 16S rRNA sequencing																		
11. Bacterial identification																		
12. Diversity of bacterial pathogens																		
13. qPCR of interesting pathogens																		
14. Prevalence of interesting pathogen																		
Part 3 Virome profile in rodents of each habitat, and phylogenetic reconstruction																		
15. Data analysis																		
16. Virome identification																		
17. Comparison of virome profile in rodents of 2 provinces																		
18. Phylogenetic reconstruction																		
19. Prevalence of interesting viruses using qPCR																		
20. Selection of candidate viruses																		
Part 4 Candidate virus with cross-species transmission potential																		
21. Retrieval of receptor binding domain sequence of candidate virus																		
22. *in sillico* receptor binding prediction model in each animal and human																		
23. Selection of potential cross-species transmitted virus based on the best docking score																		
24. Manuscript preparation and submit																		
25. Thesis defense																		

Finished tasks

Ongoing tasks

Further tasks

9. References

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