Title: Comparative Virome Study of Dengue Vector Mosquitoes in Cimahi City

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Research Objectives:

1. Assessing spatiotemporal distribution of dengue vector mosquitoes in Cimahi City.
2. Gathering Incidence Rate of dengue infection on human data in Cimahi City
3. Collecting Suspected Dengue Infection Human Blood samples in Cimahi City
4. Optimizing Molecular Steps for Dengue RNA Isolation from Mosquito and Human Samples

Methods:

1. Mosquito Samples Collection
   Sample collections were performed in every sub-district in Cimahi City (15 totals) on Dry Seasons (July – August) and Wet Seasons (December – January). Mosquito were collected using a Inhalant Mosquito Trap Indoor GM909 (Krisbow) placed each in 20 houses per sub-district in Cimahi City. The mosquito trap was placed overnight and the samples were collected next day. Collected mosquito blood samples then identified by species and sex based from its morphology. Identified mosquito were stored in microtube containing RNA Later solution and kept in –80°C freezer.

2. Human Blood Samples Collection
   Suspected Dengue Infected Human Blood Samples were collected from 13 Primary Health Care (PHC) in Cimahi City. Suspected Patients were health screened by symptoms of dengue primarily fever that lasted more than three days. After health screened, suspected patients will undergo Rapid Antigen Test for Dengue NS1 of whom the dengue positives blood will be sampled (0.5 mL to 5 mL) in EDTA Collection Tube and kept in –20°C freezer.

3. Incidence Rate of Dengue Infection on Human
   Data of Dengue Infection on Human are acquired from Cimahi City Health Department. Dengue incidence rates are recorded both on PHCs and Hospitals in Cimahi City. All of the cases then reported to Cimahi City Health Department.

4. Molecular Steps Optimization

   RNA Extraction from collected Mosquito and Human Blood samples were performed using Various Extraction Kits: Fastpure Viral DNA/RNA Minikit (Vazyme), QiaAmp Viral RNA Mini Kit (Qiagen), RNEasy Mini Kit (Qiagen), MDSEK0002 Whole Blood Nucleic Acid Extraction Reagents (Fapon Biotech) and MDSEK0001 Viral Nucleic Acid Extraction Kit (Fapon Biotech). The quality Isolated RNAs then were assayed using Microdrop Plate (Thermo Fisher) for nucleic acid quality and Qubit RNA High Sensitivity (HS) Assay and 1x dsDNA HS Assay (Thermo Fisher) for nucleic acid concentration. The dengue viral detection was also optimized of using MDE020 Dengue Virus RT PCR Reaction (Fapon Biotech).

Current Results:

1. Mosquito Sample Collections

   Mosquito collection was performed indoor inside citizen's house in each district of Cimahi City (Figure 1). This method of mosquito collection would target mosquito that inhabit houses and preferred to feed blood from human or domesticated animal. The mosquito traps were placed mostly in living room and bedroom. The owner of the houses could freely move the trap around the house as long it was turned on. Mosquito collection was performed after the mosquito trap was placed overnight.

Figure 1. Placing the mosquito trap inside the house

Mosquito population distribution in Cimahi City is dominated by *Aedes aegypti* and *Culex* mosquito (**Table 1.1**) with some of other species and genus was also detected in much smaller number such as *Ae. albopictus* and *Armigeres sp.* mosquitoes. *Ae. aegypti* tends to live and reproduce near human settlement as opposed to *Ae. albopictus* that preferred water source near vegetation and feed on farm animals.

Table 1.1. Dry Season Mosquito Sampling Data

No.	Sub-District	Ae. aegypti	Ae. Albopictus	Culex spp.	Armigeres spp.
1.	Citeureup	43	1	5	1
2.	Cipageran	75	0	9	9
3.	Cibabat	27	0	45	0
4.	Pasirkaliki	66	0	10	4
5.	Cigugur	41	0	25	0
6.	Karangmekar	27	0	2	0
7.	Baros	36	0	6	0
8.	Cimahi Tengah	33	0	22	0
9.	Padasuka	18	8	8	5
10.	Setiamanah	13	1	5	0
11.	Melong	21	0	30	0
12.	Cibeber	14	0	44	0
13.	Leuwigajah	33	0	18	0
14.	Utama	26	0	45	0
15.	Cibeureum	12	0	2	0
Total		**256**	**29**	**533**	**60**

Profile of the Cimahi City mosquito species distribution in the dry season was similar to the profile of distribution in wet season (**Table 1.2**). However, significant increase in the mosquito abundance in wet season was observed. Each sub-district in Cimahi City showed average increase 108% of mosquito abundance.

Table 1.2. Wet Season Mosquito Sampling Data

No.	Sub-District	Ae. aegypti	Ae. Albopictus	Culex spp.	Armigeres spp.
1.	Citeureup	20	12	14	17
2.	Cipageran	18	6	10	13
3.	Cibabat	12	1	14	8
4.	Pasirkaliki	53	0	4	1
5.	Cigugur	20	5	7	1
6.	Karangmekar	16	3	3	0
7.	Baros	17	2	192	0
8.	Cimahi Tengah	17	0	6	0
9.	Padasuka	7	0	22	0
10.	Setiamanah	13	0	28	0

11.	Melong	13	0	39	0
12.	Cibeber	4	0	14	20
13.	Leuwigajah	17	0	102	0
14.	Utama	18	0	32	0
15.	Cibeureum	11	0	46	0
Total		**485**	**10**	**276**	**19**

2. Human Blood Samples Collection

Human blood samples were able to be collected with the help and coordination with PHC Doctors and lab technician (**Table 1.2**). Suspected dengue patients with fever symptom were screened by Dengue NS1 Rapid Test. The all thirteen PHC in Cimahi City was spread to all sub-districts and to three districts.

Table 2.1. Suspected Dengue Infected Patients Screening

No.	Primary Health Care	Number of NS1 Test	Number of Sampled Patients	Number of Dengue Positive Samples
1	Cimahi Utara	5	5	3
2	Cimahi Selatan	32	32	15
3	Cibeber	31	19	8
4	Cibeureum	18	18	8
5	Cigugur	69	45	24
6	Cimahi Tengah	43	25	21
7	Cipageran	26	26	10
8	Citeureup	18	18	8
9	Leuwigajah	42	30	8
10	Melong Tengah	7	1	1
11	Melong Asih	40	32	6
12	Padasuka	0	0	0
13	Pasirkaliki	39	36	10
	Total	**370**	**287**	**122**

3. Dengue Incidence on Human in Cimahi City Data

The data was able to be acquired with coordination with Cimahi City Department of Health.

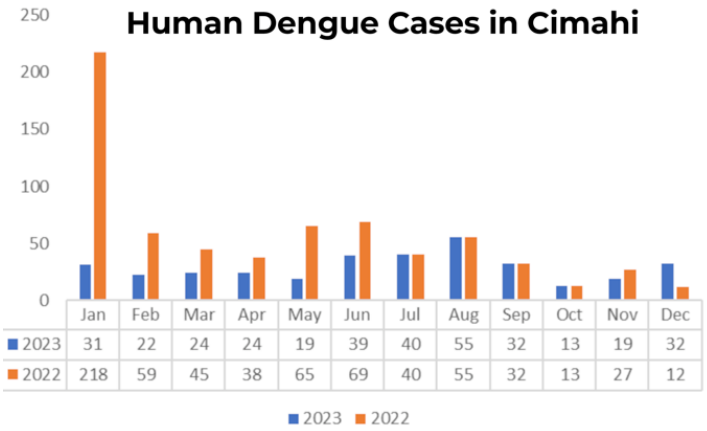

Figure 2. Dengue Incidence data in Cimahi City

Both in the wet season and dry season showed that there were peaks dengue cases in the month of July – August and December – January for the year of 2022 and 2023 (**Figure 2**).

4. Optimization of Molecular Detection of Dengue

Using various RNA/DNA Extraction kit, molecular steps were optimized to choose the best kit for all type of samples. Based on the results of **Table 4.1**, the reagents chosen for extraction are Vazyme Fastpure Viral RNA/DNA. The Decision is based on purity, concentration, and final elution volume.

Table 4.1. Extraction Reagents Optimizations

No.	Kit Name	Extraction type	RNA conc. (ng/uL)	Volume	260/280	260/230
1.	RNEasy Mini Kit	Centrifuge column	10 – 40	60 uL	~2,00	~2,10
2.	Vazyme Viral DNA/RNA Kit	Centrifuge column	20 – 40	50 uL	1,95 – 2,05	~2,20
3.	QiaAmp Viral RNA	Centrifuge column	1 – 10	100 uL	~2,00	~2,10
4.	Fapon Viral Nucleic Acid	Magnetic beads	1 – 10	60 uL	1,95 – 2,05	~2,20
5.	Fapon Wholeblood Nuclei Acid	Magnetic beads	1 – 10	60 uL	1,90 – 2,10	~2,20

Future Challenges:

- All of the samples must be retained for all research objectives e.g. RT-PCR, metaviromics, and phylogenetic analysis.
- Samples extracted with Vazyme Fastpure Viral RNA/DNA must be purified using DNAse or RNAse to acquire specified type Nucleic Acid (RNA or DNA).
- The Dengue Incidence data must be validated with 2024 and 2025 data to check whether there is a peak shift of the dengue cases each year.