Characteristic differences between breeding places with and without *Culex sp.* larvae on lymphatic filariasis patient surroundings in an endemic area

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Abstract. Brebes Regency is one of the Lymphatic Filariasis (LF) endemic areas in Central Java Province, Indonesia. Ketanggungan Subdistrict, Bantarkawung Subdistrict and Paguyangan Subdistrict have the highest case numbers. *Culex sp.* is one of the vectors of LF in Java, Indonesia. This study was conducted to obtain information about the characteristics of the breeding places for *Culex sp.* in the area where people with LF live. The finding could be used as information with facts for society. It was a descriptive quantitative study with an observational design. The observation was conducted in a radius of 200 meters around the settlements of LF (15 patients) in three sub-districts in the Brebes Regency. Observed and measured data were larval density, water acidity, air temperature, air humidity, water flow, prominent flora presence, and prominent fauna presence. The breeding places observed were puddles, rivers, and sewers. Puddles and sewers with no aquatic animals were observed positively for the presence of *Culex sp.* larvae. The most preferred breeding place for *Culex sp.* in the study area were uncovered sewers.

1. Introduction

Lymphatic Filariasis (LF) is one of the Neglected Tropical Diseases (NTDs), which still is a global public health problem. LF does not cause lethality but causes disability because of the swelling on some body parts like legs, hands, scrotum, and breasts. It leads to unproductivity and social exclusion for the patients [1,2]. Central Java Province, Indonesia, in 2018 had increased the number of LF endemic areas from 2 to 9 areas. Brebes Regency was one of the new LF endemic areas in Central Java Province, Indonesia [3]. Ketanggungan Subdistrict, Bantarkawung Subdistrict and Paguyangan Subdistrict have the highest LF case numbers in Brebes Regency.

COVID-19 pandemic adds disadvantages to the LF elimination program. LF elimination is a global program from WHO (World Health Organization), also applied in Indonesia. Mass Drug Administration (MDA) of LF, the main effort of the LF elimination program, could not be held simultaneously as the COVID-19 vaccination program. It causes an increasing impact in LF case numbers [4–6]. Therefore, another effort should be taken to keep the LF elimination program making progress.
Vector control with environmental management is another effort that could be taken for mitigation of MDA postponement due to the COVID-19 pandemic. The vector of LF is various mosquitoes (Culex sp., Aedes sp., Anopheles sp., Mansonia, and Armigeres). Previous studies proved the environment's contribution to LF transmission by mosquitoes [3,7–14]. Environmental management for vector control is also resolvable for insecticide resistance in mosquitoes [15–18].

Culex sp. is one of the LF vectors [19] and the primary vector of LF in Java Island. Female Culex sp. lays eggs commonly on stagnant water surfaces. About 300 eggs would form a flock and float on the water surface, then be larvae in 48 hours. The larvae would grow up to be the adult mosquito in 7-8 days [20,21]. Eggs and larval phases are the weakest phases in the Culex sp. life cycle. Those phases are easier to control without difficulty in handling flying mosquitoes. The larval phase is more accessible to identify by ordinary people than the eggs phase. Society could make simple environmental management efforts to control Culex sp. larvae.

This study was conducted to obtain information about the characteristics of the breeding places for Culex sp. in the area where people with LF live in Ketanggungan Subdistrict, Bantarkawung Subdistrict and Paguyangan Subdistrict. Furthermore, the main characteristics that distinguish between breeding places with and without Culex sp. larvae will be found. The finding could be used as information with facts for society. Adequate information with objective facts on society's surroundings is expected to build last long behaviour changes in environmental management [22,23].

2. Methodology
It was a descriptive quantitative study with an observational design. The observation was conducted in a radius of 200 meters around the settlements of all lymphatic filariasis (LF) patients (15 patients) in three sub-districts in Brebes Regency, namely Ketanggungan Sub-districts, Bantarkawung Sub-districts and Paguyangan Sub-districts. The three sub-districts were selected due to their highest lymphatic filariasis case in Brebes Regency, Indonesia. Data of LF patients were collected from Public Health Centers and cadres of the three sub-districts, with the permission of Brebes Regency Health Office. The observation was conducted by surveying and measuring the sites where Culex sp. probably bred. Larval density was estimated by calculating the average of total larvae number from all taken dips. 10 dips were taken from every observed site using dipper (volume 350 mL). Larval density was categorized as “high” when it was more than 20 larvae/dip. Distance between patient house coordinate and the observed site coordinate was measured using GPS and Google Earth. Water acidity was measured with pH indicator strips. Air temperature and humidity were measured with a thermohygrometer. Water flow, prominent flora presence, and prominent larvae presence were observed with direct observation. Data were presented quantitatively in tables. Data analysis was done univariately in paragraphs presenting the founding and making an interpretation about LF transmission risk. This study was conducted in July-September 2020 after being registered by the Health Ethics Committee of Semarang State University (Universitas Negeri Semarang). This study has obtained an ethical approval recommendation letter with No. 134 / KEPK / EC / 2020.

3. Results and discussion
The observation was conducted in a radius of 200 meters around the settlements of all lymphatic filariasis (LF) patients (15 patients) in three sub-districts in Brebes Regency, namely Ketanggungan Sub-districts, Bantarkawung Sub-districts and Paguyangan Sub-districts. Table 1 presents the characteristics of LF in the patients. The most LF case was on females (73.3%) and 50-70 (60.0%). People with these characteristics were mostly spent their time at home that highly possibly bitten by mosquitoes from surrounding.

Table 1. Characteristics of lymphatic filariasis patients.

| Patient Code | Age | Gender | Address          |
|--------------|-----|--------|-----------------|
| 1            | 40  | F      | Ketanggungan, RT 06 / RW 04 |
| 2            | 61  | M      | Ketanggungan, RT 06 / RW 05 |
There were 29 sites observed in a radius of 200 m from patient's houses consisted of puddles, rivers, and sewers. The inundation observed consisted of puddles of groundwater, rice fields, ponds, and puddles on unused items. 8 patients live near the river. The location of the river observed is directed at the part of the river that is closest to the patient's house. The sewers observed consisted of soil-based sewers, plastered sewers, closed sewers, and open sewers. Some pictures of them are displayed in figure 1. Distribution in cent of breeding places and Culex sp. larvae presence on LF patients’ surroundings were delivered in table 2. The details of those sites are presented in table 3.

![Figure 1](image)

**Figure 1.** (a, b, c). river, (d). soil-based puddle, (e). half-covered and uncovered plastered sewer, (f). puddle on an unused item, (g). rice field puddle, (h). pond puddle, (i). uncovered plastered sewer, (j). soil-based sewer.

**Table 2.** Distribution of breeding places and Culex sp. larvae presence on LF patient’s surroundings.

| Breeding place | With larvae | Without larvae | Total |
|----------------|-------------|----------------|-------|
|                | f           | %              | f     | %    |
| Puddle         | 3           | 42.9           | 4     | 57.1 |
| River          | 0           | 0.0            | 8     | 100.0|
| Sewers         | 7           | 50.0           | 7     | 50.0 |
|                | 10          | 100.0          | 19    | 100.0|

| Breeding place | With larvae | Without larvae |
|----------------|-------------|----------------|
|                | f           | %              |
| Puddle         | 3           | 30.0           |
| River          | 0           | 00.0           |
| Sewers         | 7           | 70.0           |

| Breeding place | With larvae | Without larvae |
|----------------|-------------|----------------|
|                | f           | %              |
| Puddle         | 3           | 4             |
| River          | 0           | 8             |
| Sewers         | 7           | 7             |
There is no *Culex sp.* larvae presence in all observed river sites. Garbage was found in all of the observed river sites. Most of them were cloudy, stagnant, pH 7-8, air temperature 22.83-26.63°C, and humidity 73.95-88.58%. Tallan & Mau (2016) found that pH 7.2-7.7 was optimum for *Culex sp.* larvae, whereas Deme et al. (2017) proved that *Culex sp.* larvae could survive in various pH and polluted water with low dissolved oxygen [24,25]. *Culex sp.* larvae grow optimum at temperature 23-27°C and humidity 60-80% [26,27]. Those conditions on observed river sites were suitable for *Culex sp.* larvae, but on the contrary, there were no *Culex sp.* larvae found on the 8 river sites. The high volume of water, the vast area of the river, and the presence of river stones probably set the larvae hidden and not accessible. The river sites had 2-5 m width, pH 7-8, and generally had various aquatic flora and fauna. Fish and other prominent fauna (crab and tadpole) mainly were observed in river sites (62.5%). It indicated that the quality of river water was good enough for them to live. Suitable water quality for fish to live was contrary, not suitable for *Culex sp.* larvae. Mosquito larvae preferred septic conditions where dissolved oxygen is 0-2, which is unsuitable for fish to live [28]. The presence of fish indicated that the river zone was not in septic condition. It was the most logical reason for *Culex sp.* larvae absence in the observed river sites. Moreover, fish itself could be mosquito larvae, predators. Similar conditions were also found in the other sites without *Culex sp.* larvae. Puddles and sewers with no *Culex sp.* larvae mainly were garbage, stagnant, cloudy, pH 6-8, air temperature 22.08-25.91°C, humidity 73.95-88.58%, and had fish presence.

There were ten sites with *Culex sp.* larvae presence, 70% were sewers, and 30% were puddles. The sewers of the sites were uncovered and 90% stagnant. The puddles of the sites were a puddle on the unused box and soil-based puddles. The sites' water was 80% stagnant, 90% with garbage, 100% cloudy and muddy, pH 6-8, air temperature 23.71-27.57°C, and humidity 68.00-81.08%, which were suitable for *Culex sp.* larvae growth [24–27]. No prominent fauna present in the sites with *Culex sp.* larvae presence. It indicated that water quality in the sites had reached septic conditions where dissolved oxygen was low and polluted, which was not suitable for fish to live. This septic condition was suitable for *Culex sp.* larvae growth [28]. Moreover, no fish meant no predator for the larvae.

There were 2 sites with a larval density of more than 20 (high density). It indicated that *Culex sp.* preferred the sites to lay eggs and also suitable for *Culex sp.* larvae growth. *Culex sp.* prefers to lay eggs on the stagnant water surface [20]. The larvae need suitable physicochemical characteristics on the water to grow up [25,29–31]. The higher larval density, predictably, the more significant survived adult *Culex sp.* number will be. The more significant survived adult *Culex sp.* number, the higher LF transmission risk. However, larval density was not the only factor to influence survived adult *Culex sp.* number. The overcrowded habitat of *Culex sp.* larvae would result in delayed development to be adult mosquitoes [32]. The distance between sites with *Culex sp.* larvae and LF patients’ house were within 200 meters. LF patients had filarial worm in their blood. They were the source of filarial worm. In other side, *Culex sp.* could fly in radius 200 meters, even recorded could reach2 miles (3.2 km) [20,33]. It is important to prevent *Culex sp.* larval presence in breeding places around the patients’ dwelling. Preventing the sites from garbage could be done to keep the stream flowing and keep the water quality to protect the presence of aquatic animals as larva predators. It is also necessary to have the sewers covered to prevent mosquitoes breed on them quickly. Health officers, together with health cadres, were expected to educate society about it. It would be probably a long-term effort, but previous studies have proved its success [23,34].
Table 3. Characteristics of breeding places with and without Culex sp. larvae on LF patient surroundings.

| Breeding Places with Culex sp. Larvae | Breeding place | Surrounded patient code | Air temperature (°C) | Air humidity (%) | Garbage presence | Phys-soc turbidity | Water flow | Distance from patient's house (m) | pH | Prominent flora presence | Prominent fauna presence | Larval density |
|---------------------------------------|----------------|-------------------------|----------------------|------------------|------------------|-------------------|------------|--------------------------------|----|-----------------------|-------------------|--------------|
| Puddle                               | 3              | 26.34                   | 73.95                | Yes              | Cloudy           | Stagnant          | 142.08     | 6                              | 7  | Algae                 | -                 | 5.7          |
|                                       | 4              | 25.00                   | 81.08                | No               | Cloudy           | Stagnant          | 42.30      | 7                              | -  | -                     | -                 | 1.9          |
|                                       | 15             | 26.63                   | 74.42                | Yes              | Cloudy           | Flowing           | 193.13     | 7                              | Algae | -                     | -                 | 2.0          |
| Sewer                                | 1              | 25.91                   | 75.30                | Yes              | Muddy            | Stagnant          | 200.00     | 7                              | -  | -                     | *38.3             | 19.2         |
|                                       | 3              | 26.34                   | 73.95                | Yes              | Cloudy           | Stagnant          | 200.00     | 7                              | -  | -                     | -                 | 2.7          |
|                                       | 4              | 25.00                   | 81.08                | Yes              | Cloudy           | Stagnant          | 192.30     | 8                              | Algae | -                     | -                 | 8.5          |
|                                       | 5              | 27.57                   | 74.75                | Yes              | Cloudy           | Stagnant          | 173.40     | 8                              | -  | -                     | -                 | 19.2         |
|                                       | 6              | 27.34                   | 68.00                | Yes              | Cloudy           | Stagnant          | 200.00     | 7                              | Algae | -                     | -                 | 19.2         |
|                                       | 7              | 23.71                   | 76.21                | Yes              | Cloudy           | Stagnant          | 19.30      | 8                              | -  | -                     | -                 | 13.1         |
|                                       | 15             | 26.63                   | 74.42                | Yes              | Cloudy           | Flowing           | 100.00     | 7                              | Algae | -                     | -                 | *53.4        |
| Breeding Places without Culex sp. Larvae | 2              | 25.91                   | 75.30                | No               | Cloudy           | Stagnant          | 53.40      | 7                              | -  | Fish                  | -                 | -            |
|                                       | 9              | 24.30                   | 78.92                | No               | Clear            | Stagnant          | 130.70     | 7                              | -  | -                     | -                 | -            |
|                                       | 11             | 22.83                   | 88.58                | Yes              | Cloudy           | Stagnant          | 96.60      | 6                              | -  | -                     | -                 | -            |
|                                       | 13             | 25.50                   | 78.75                | Yes              | Cloudy           | Stagnant          | 200.00     | 7                              | -  | -                     | -                 | -            |
| River                                 | 1              | 25.91                   | 75.30                | Yes              | Cloudy           | Stagnant          | 22.50      | 8                              | -  | -                     | -                 | -            |
|                                       | 2              | 25.91                   | 75.30                | Yes              | Cloudy           | Stagnant          | 145.50     | 7                              | Fish | -                     | -                 | -            |
|                                       | 3              | 26.34                   | 73.95                | Yes              | Cloudy           | Stagnant          | 94.51      | 8                              | Grass | Fish                  | -                 | -            |
|                                       | 7              | 23.71                   | 76.21                | Yes              | Cloudy           | Stagnant          | 85.60      | 8                              | -  | -                     | -                 | -            |
|                                       | 8              | 24.42                   | 86.25                | Yes              | Cloudy           | Stagnant          | 26.80      | 7                              | Algae | Crab                  | -                 | -            |
|                                       | 10             | 22.83                   | 88.58                | Yes              | Cloudy           | Stagnant          | 28.40      | 7                              | -  | -                     | -                 | -            |
|                                       | 11             | 22.83                   | 88.58                | Yes              | Cloudy           | Stagnant          | 20.40      | 8                              | Kale | Tadpole                | -                 | -            |
|                                       | 15             | 26.63                   | 74.42                | Yes              | Clear            | Flowing           | 45.30      | 7                              | Algae | Fish                  | -                 | -            |
| Sewer                                 | 8              | 24.42                   | 86.25                | Yes              | Cloudy           | Stagnant          | 200.00     | 8                              | Algae | -                     | -                 | -            |
|                                       | 9              | 24.30                   | 78.92                | Yes              | Cloudy           | Stagnant          | 200.00     | 8                              | -  | Fish                  | -                 | -            |
|                                       | 10             | 22.83                   | 88.58                | Yes              | Cloudy           | Stagnant          | 100.00     | 8                              | -  | -                     | -                 | -            |
|                                       | 11             | 22.83                   | 88.58                | Yes              | Cloudy           | Stagnant          | 100.00     | 8                              | -  | -                     | -                 | -            |
|                                       | 12             | 22.08                   | 84.83                | Yes              | Cloudy           | Flowing           | 100.00     | 8                              | -  | Fish                  | -                 | -            |
|                                       | 13             | 25.50                   | 78.75                | Yes              | Cloudy           | Flowing           | 200.00     | 7                              | -  | Fish                  | -                 | -            |
|                                       | 14             | 25.50                   | 78.75                | Yes              | Cloudy           | Stagnant          | 100.00     | 8                              | -  | -                     | -                 | -            |

*High larval density
4. Conclusion
The main differentiating character between the breeding place with and without Culex sp. larvae was prominent fauna presence. No prominent fauna presence in a breeding place with Culex sp. larvae, whereas fish, tadpole, and crab were found in breeding places without Culex sp. larvae. Preventing the sites from garbage is essential to keep the stream flowing and keep the water quality at the allowed level, which is expected to protect the presence of aquatic animals as larvae predators.

This study found that the most preferred breeding places for Culex sp. in the study area were uncovered sewers. Uncovered sewers had the highest Culex sp. larval density with appropriate physicochemical characteristics. It was a potential risk of LF transmission since the distance between the uncovered sewers and the patient's house was 200 meters. LF patients are filarial worm sources that are disease agents. It is essential to control larval density around patients' dwellings. It is necessary to have the sewers covered to prevent mosquitoes breed on them quickly.

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