Characteristics of *Plasmodium knowlesi* Malaria based on the results of PCR examinations in Aceh Besar District

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**Abstract.** Malaria is a disease that conducted the health problems in Indonesia. Human malaria is caused by Plasmodium. There are 5 types of plasmodium, which were known to be able to infect humans, namely Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, and Plasmodium knowlesi. The increasing of malaria cases due to Plasmodium knowlesi in Aceh Besar District for the period 2019-2020 be the fundamental of this research related to the proportion of patient characteristics, which include: sex, age, occupation, domicile and health facilities that carry out examinations and provide treatment and the connection with the PCR examination results. This research applied a descriptive method with cross-sectional design. The total sample were 39 samples with a total positive PCR sample were 16 samples that consist 81.3% of male gender (13 people), 62.5% of adult (10 people), 56.3% of worker (9 people), 75% of native (12 people) and 81.3% of the people from Lembah Seulawah health center (13 people). The characteristics patients infected by Plasmodium knowlesi malaria in the Aceh Besar District are: Male, adult, working, native population and the highest number were in the Lembah Seulawah Health Center.

1. **Introduction**

Malaria is a disease transmitted by the bite of a female mosquito that contains parasites from the group of *protozoan*, *Plasmodium*. In general, the types of mosquitoes that are vectors of malaria are from the genus *Anopheles* [1]. Currently, this disease is still one of the main health problems in Indonesian society. In 2017, there are 261,271 positive cases were reported. This number increased from 2016 which only amounted to 218,450 cases [2,3].

The types of plasmodium that are known and can infect humans are: (1) *Plasmodium vivax*, (2) *Plasmodium falciparum*, (3) *Plasmodium malariae*, (4) *Plasmodium ovale* and (5) *Plasmodium knowlesi* [4,5]. The malaria parasite life cycle requires two hosts, on the four types of plasmodium apart from *Plasmodium knowlesi*. The two hosts are female and human *Anopheles* mosquitoes. Meanwhile, the *Plasmodium knowlesi* cycle in human hosts is still being studied [4]. *Plasmodium knowlesi* is a zoonotic parasite from primates that is transmitted through mosquito bites, and currently, *P. knowlesi* is recognized as the fifth type of plasmodium parasite capable of infecting humans [5],[6].
Aceh Province reported that in 2017 there were 56 suspected of P. knowlesi, this number increased in 2018 to 86 people [7]. Since 2019, the Laboratory of the Aceh Research and Development Center has been active as one of the P. knowlesi malaria confirmation laboratory networks. The number of P. knowlesi malaria suspected that entered the laboratory in 2019 was 19 samples from Aceh Singkil (1 person), Aceh Utara (1 person), Aceh Jaya (7 people), Aceh Tengah (1 person), Aceh Selatan (6 people), Aceh Besar (2 people) and Aceh Barat (1 person) [8]. For Sabang area itself until the end of November 2019, there were 6 people who had tested positive for P. knowlesi malaria.

The diagnosis of P. knowlesi using microscopic examination is often inaccurate. This is widely reported in Asian countries. This is because it is similar to Plasmodium falciparum and Plasmodium malariae. Therefore the examination method using the PCR method is preferred because it is more accurate [9]. The aim of this study was to see the proportion of sex, domicile, age category, occupation category and health center visited by P. knowlesi malaria suspects in Aceh Besar district and its relation to PCR results.

2. Materials and Methods
The type of this research was descriptive with cross-sectional design. The data were taken from the PCR examination section of P. knowlesi at the Aceh Research and Development Center. The research data were patients who were subjected to the examination of filter paper samples among 30 August 2019 to 26 March 2020. The Patients were grouped into 4 age groups according to the WHO category, namely, children (≤ 10 years), adolescents (11-19 years), adults (20-60 years), and elderly (≥61 years). The domicile is categorized from the patient's residential address, while the occupation is based on working and non-working.

The sample used is the total sample. Positive determination of P. knowlesi using the single step PCR method by [10]. All data will be presented in tabular form and analysed using bivariate analysis to observed the proportion of characteristics on suspect and patient malaria P.knowlesi.

3. Results and Discussion
The results of the data obtained from the PCR sample examination section, the total sample was 45 samples, and those that met the data completeness criteria were 39 samples. The results of the bivariate analysis are presented in Table 1. From Table 1, the number of patients suspected of P. knowlesi was male as many as 31 people (79.5%), most of which were also found in the male gender, namely 13 people (81.3%). In the study conducted by Wesolowski et al in 2015 showed that men were more exposed to P. knowlesi malaria [11]. This is because the activities of men as workers / breadwinners are required to work outside the home so that the risk of being bitten by mosquitoes is higher and also the work that leads to activities close to forest areas such as logging, construction / development, mining and so on as presented in Table 2 [12].

| Gender     | PCR positive | PCR negative | Total   |
|------------|--------------|--------------|---------|
| Male       | 13 (81.3%)   | 18 (78.3%)   | 31 (79.5%) |
| Female     | 3 (18.8%)    | 5 (21.7%)    | 8 (20.5%)  |
| Total      | 16           | 23           | 39       |

| Domicile           | PCR positive | PCR negative | Total   |
|--------------------|--------------|--------------|---------|
| Indigenous people  | 12 (75.0%)   | 18 (78.3%)   | 30 (76.9%) |
| Indigenous people  | 4 (25.0 %)   | 5 (21.7%)    | 9 (23.1%)  |
| Total              | 16           | 23           | 39       |
Domicile/place of residence for the suspects and positive confirmation of *P. knowlesi* were a native population of 30 people (76.9%) and positive as many as 12 people (75%). The area where the positive confirmed case of *P. knowlesi* was located in the area bordering the forest and there were many long-tailed macaques (*Macaca fascicularis*) and also caused by activities or work in the forest area [12].

### Table 3. Categories of age and PCR results

| Age      | PCR                      | Total |
|----------|--------------------------|-------|
|          | positive | negative |       |
| Children | 3 (18.8%) | 2 (8.7%) | 5 (12.8%) |
| Adolescent | 2 (12.5%) | 7 (30.4%) | 9 (23.1%) |
| Adult    | 10 (62.5%) | 14 (60.9%) | 24 (61.5%) |
| Ederly   | 1 (6.3%) | 0 (0.0%) | 1 (2.6%) |
|          | 16       | 23       | 39     |

The age category of *P. knowlesi* suspected was 24 adults (61.5%) with the highest number of positive confirmed cases in adults as many as 10 people (62.5%). This was in line with Asmara's (2018) statement which stated that *P. knowlesi* malaria occurs in adults and only about 10% of children are reported to be [13].

### Table 4. Occupation catagories and PCR result

| Works      | PCR                      | Total |
|------------|--------------------------|-------|
|            | positive | negative |       |
| Works      | 9 (56.3%) | 13 (56.5%) | 22 (56.4%) |
| Not working | 7(43.8 %) | 10 (43.5%) | 17 (43.6%) |
| Total      | 16       | 23       | 39     |

The suspected of *P. knowlesi* for occupation category was 22 people (56.4%) and 9 people (56.3%) was confirmed positive cases. The jobs confirmed as positive cases are mostly working as forest workers, building towers near forest areas and farmers who cultivate fields around the forest. This is consistent with the statement of Abegunde (2004) that said, the incidence of *P. knowlesi* malaria is caused by working or doing activities around forest areas where long-tailed macaques (*Macaca fascicularis*) are found [12].

### Table 5. Health Center categories and PCR results

| Health facilities | PCR                      | Total |
|-------------------|--------------------------|-------|
|                   | positive | negative |       |
| Kota Cot Gli      | 1 (6.3%) | 0 (0.0%) | 1 (2.6%) |
| Krueng Barona Jaya | 1 (6.3%) | 0 (0.0%) | 1 (2.6%) |
| Lhoong            | 1 (6.3%) | 0 (0.0%) | 1 (2.6%) |
| Lembah Seulawah   | 13 (81.3%) | 23 (100%) | 36 (92.3%) |
| Total             | 16       | 23       | 39     |

The health center category for the number of *P. knowlesi* malaria suspects was found in the Lembah Seulawah Health Center as many as 36 people (92.3%), of which 13 people (81.3%) were confirmed positive cases. The existence of the health center where the suspect got the examination / treatment was the forest areas, namely the Lembah Seulawah, Lhoong and Kota Cot Gli health centers based on the Google map satellite data tracked by the author. This was in line with several studies which stated that the incidence of *P. knowlesi* malaria occurs due to close interaction with the forest and the presence of long-tailed macaques (*Macaca fascicularis*) [12,14–16].
4. Conclusions

The characteristics of *P. knowlesi* malaria suspects and patients in the Aceh Besar district were: the majority are male, adult age, work status, indigenous people and the highest number of suspects and patients / positive confirmed cases are in the health facilities of the Lembah Seulawah community Health Center where positive case findings are always related to activities and residential areas adjacent to forest areas.

References

[1] Kemenkes RI 2016 Pusat Data Dan Informasi Penyakit Malaria 2016 1–7.
[2] Pusdatin K R 2017 *Data dan Informasi Profil Kesehatan Indonesia 2016* (Jakarta, Indonesia).
[3] Pusdatin K R 2018 *Profil Kesehatan Indonesia Tahun 2017* (Jakarta, Indonesia).
[4] Menteri Kesehatan RI 2013 *Permenkes RI No.5 Tahun 2013 Tentang “Pedoman Tata Laksana Malaria”* (Indonesia).
[5] Abeyasinghe R 2016 *Plasmodium knowlesi* current status and the request for review by an Evidence Review Group *Plasmodium knowlesi Curr. status Req. Rev. by an Evid. Rev. Gr.*
[6] Preis J and Lutwick L 2014 *Plasmodium knowlesi* The Epidemiology of *Plasmodium vivax*
[7] Tim P2P Dinkes Aceh 2019 *Laporan Bulanan Penemuan dan Pengobatan Malaria di Puskesmas Aceh*.
[8] Tim Balai Litbangkes Aceh 2019 *Laporan Hasil Pemeriksaan Plasmodium knowlesi di Balai Litbangkes Aceh Tahun 2019*.
[9] Suhandi D A and Suwandi J F 2017 *Medula* 7 (5) 177–182.
[10] Imwong M, Tanomsing N, Pukrittayakamee S, Day N P J, White N J and Snounou G 2009 *J. Clin. Microbiol.* 47 4173–75.
[11] Wesolowski R, Wozniak A, Mila-Kierzenkowska C and Szewczyk-Golec K 2015 *Korean J. Parasitol.* 53 575–581.
[12] Abegunde A T 2004 *Lancet* 364 1217.
[13] Asmara I G Y 2018 *J. Penyakit Dalam Indones.* 5 200–208.
[14] Ahmed M A and Cox-Singh J 2015 *ISBT Sci. Ser.* 10 134–40.
[15] Antinori S, Galimberti L, Milazzo L and Corbellino M 2013 *Acta Trop.* 125 191–201.
[16] Ong C W M, Shir Y L, Wee H K, Ooi E E and Tambyah P A 2009 *Am. J. Trop. Med. Hyg.* 80 927–928.