Dominance of *Anopheles maculatus* over Etawa Crossbred Goats Population in Malaria Pre-Elimination Areas

Dominansi *Anopheles maculatus* Pada Populasi Ternak Kambing Peranakan Etawa di Daerah Pre-Eliminasi Malaria

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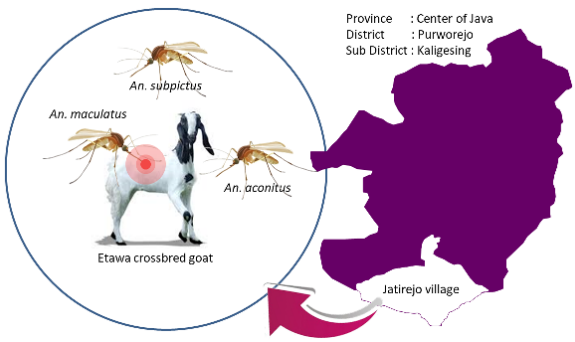
ABSTRACT

Malaria disease is transmitted by the Anopheles sp. vectors. The climate and environmental changes will in fact affect the life and bionomic vector of Anopheles sp. The Anopheles species in an area are strongly influenced by the availability of brood stocks. Such efforts to control malaria have to be in line with controlling the Anopheles population in that area. Information on the behaviors, bionomic characteristics, and habitat preferences of Anopheles species is thus needed. It is very important to select a control program according to the types of vectors to be controlled. This study aimed to examine the dominance of Anopheles species in malaria-endemic areas with currently pre-elimination status with the Etawa goats population. A field survey was necessarily conducted to catch mosquitoes during nighttime. Catching using human bait and livestock bait started from 06.00 pm to 04.00 am. Identification of species morphology used the WRBU guidelines. The catch results were dominated by Anopheles (63.64%), followed by Culex (27.27%) and Aedes (9.09%), respectively. The caught Anopheles consisted of An. maculatus (64.29%), An. subpictus (28.57%) and An. aconitus (7,14%) respectively. It was found that An. maculatus type surprisingly still dominated the mosquito population in the malaria pre-elimination area in Purworejo. Thereby, it is important to develop such a malaria control method based on mosquito attack diversion because the dominant vector in this area is more zoophilic.

ABSTRAK

Penyakit malaria ditularkan oleh vektor *Anopheles sp*. Terjadinya perubahan iklim dan lingkungan akan mempengaruhi kehidupan dan bionomik vektor *Anopheles sp*. Jenis Anopheles di suatu daerah sangat dipengaruhi oleh ketersediaan jenis perindukan. Upaya pengendalian malaria harus sejalan dengan pengendalian populasi vektor Anopheles sp di suatu daerah. Informasi tentang perilaku, karakteristik bionomik, dan preferensi habitat spesies *Anopheles sp* sangat diperlukan. Penting untuk memilih program pengendalian sesuai jenis vektor yang akan dikendalikan. Penelitian ini bertujuan untuk mengkaji dominansi spesies Anopheles di daerah endemis malaria yang saat ini dalam status pre-eliminasi dengan populasi kambing Etawa. Survei lapangan dilakukan untuk menangkap nyamuk pada malam hari. Penangkapan menggunakan umpan manusia dan umpan ternak dimulai dari pukul 18:00 - 04:00. Identifikasi morfologi spesies menggunakan pedoman WRBU. Genus Anopheles mendominasi tangkapan (63,64%) diikuti oleh *Culex vishnui* (27,27%) dan *Aedes albopictus* (9,09%). Kelompok Anopheles yang tertangkap terdiri dari *An. maculatus* (64,29%), *An. subpictus* (28,57%) dan *An. aconitus* (7,14%). *An. maculatus* masih mendominasi populasi nyamuk di daerah pra-eliminasi malaria di Purworejo. Penting untuk mengembangkan metode pengendalian malaria berbasis pengalihan serangan nyamuk karena vektor yang dominan di daerah ini lebih bersifat zoofilik.

GRAPHICAL ABSTRACT



Keyword
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INTRODUCTION

The data on mosquito diversity are considered valuable to consider with regard to vector-borne disease control programs (Muhammad et al., 2015). Each type of mosquito with a certain species has certain unique behaviors and habitats, making it distinct from other species (Setiyaningsih et al., 2015). Certain areas with the incidence of malaria are connected with the data and information regarding the presence, characteristics, and bionomics of the Anopheles mosquito vector (Muhammad et al., 2015; Muchid et al., 2015; Rahmawati et al., 2014). This is related to the prevention of disease transmission through female Anopheles mosquitoes. It is also related to vector population control efforts that cannot ignore the natures and behaviors of Anopheles life (Windyaraini et al., 2020).

The number of malaria cases in Central Java Province has been gradually declining since 2016, with a total of 1,140 cases downgrading to only 273 cases in 2020 in the third quarter. The Annual Parasitic Incidence (API) value also followed from 0.03 in 2016 down to 0.008 per 1,000 population in 2020. This achievement far exceeded the API target in 2020 of 0.06 per 1,000 population. The proportion of indigenous cases was smaller as compared to imported cases. In 2019, 0.5% of indigenous cases occurred, and in 2020 all malaria cases were categorized as imported cases (Wibowo, 2020).

Purworejo regency has been concluded as a malaria-endemic area for years, especially in the Bukit Menoreh. Until 2020, there were still six imported cases (Wibowo, 2020). One of the malaria-endemic areas in Purworejo is namely Jatirejo village. It has been relatively difficult for the city to keep away from its endemic status for decades. In 2018, indigenous cases could be reduced to zero incidents, and malaria-endemic status could thus be released (Bayu, 2018). The existence of imported cases and the anopheles population is very important. Some local anopheleses were confirmed as a malaria vector (Enny et al., 2007). The presence of imported cases is getting serious treatment. It can trigger the new transmission of local cases (Prabowo et al., 2019).

Various physical factors will influence the types and varieties of mosquitoes in an area. Adult vectors are found more frequently and at higher densities in cattle sheds than in human dwellings (Thomas et al., 2017). Anopheles populations in cattle pen areas are higher than in residential areas, which accounted for 62.8% An arabinoses is identified as containing animal-sourced blood feed (Eba et al., 2021). Out of the total of 16,468 Anopheles females, 2723 specimens (16.54%) were collected from humans and 13,745 (83.46%) captured from cattle (Tananchai et al., 2019). The existence of breeding places is an important thing to be considered (Muhammad et al., 2015). Relatively high temperature and humidity, with hilly topography, natural forest with mountain streams flowing and fields, is considerably a living habitat for several species of Anopheles mosquitoes (Taviv et al., 2015). Vector control carried out is called Indoor Residual Spraying (IRS) and the use of insecticide-treated mosquito nets. The

bioassay test results on the effectiveness of the IRS application on a maculatus mosquito showed that after one month, the application was ineffective. Effectiveness testing of mosquito nets usage for three months is still effective in killing *An. maculatus* mosquito (Setiyaningsih et al., 2018). Unfortunately, no data shows the Anopheles vector population after the control program implementation. This study aimed to determine both the presence and the dominance of the anopheles mosquito found in the malaria pre-elimination area over Jatirejo Village, Kaligesing District, Purworejo Regency. It is expected that such a current study can offer a solution to preventing the transmission of malaria.

METHODS

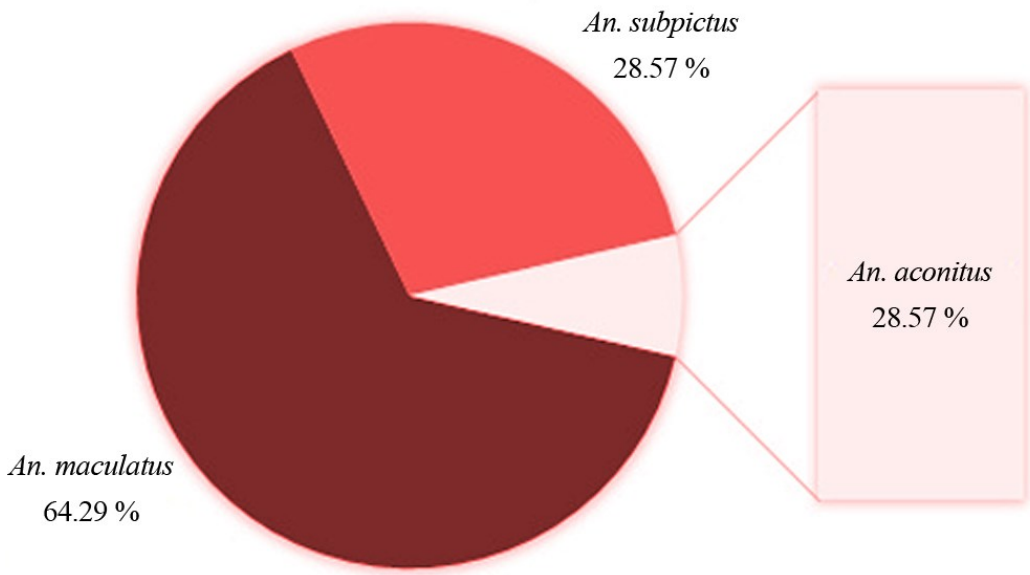
An observational study was conducted to explore the anopheles population in the Etawa crossbreed goat shed area. The arrests were made at 50 sampling points. The research sample was all mosquitoes caught using the human and livestock bait method. The sampling point was determined following the presence of each resident's cattle pen while catching using people bait was carried out in the vicinity of the cage.

The survey was held in Jatirejo Village, Kaligesing District, Purworejo Regency. This location is in the Menoreh hill area, which has long been a major malaria-endemic area in Central Java and Yogyakarta Special Region Province. At the end of 2018, there were no reported local cases of malaria; however, there were still imported cases until 2020. Currently, it is in a pre-elimination status and is being implemented as a complete program for malaria cases. The environmental conditions of the research location are very supportive as a breeding ground for Anopheles. There are a lot of holes in the ground in various places. This hole has been caused by hilly natural conditions and uneven ground surface contours. In addition, the footprints of large livestock were found around the cattle pens. These soil holes will be filled with water during the rainy season and potentially become Anopheles' breed.

The mosquito survey was conducted at the start of the rainy season in October. The catch of mosquitoes utilized human bait and livestock bait (Ndiath et al., 2011; Wong et al., 2013; Laurent et al., 2016; Kenea et al., 2017). The volunteers for human bait were a group of local-village-malaria officers who had received prophylaxis for malaria, while the livestock bait used goats in the resident's pens. Catching mosquitoes using an aspirator (Muhadi, 2013) was conducted from 6.00 pm to 04.00 am. The caught mosquitoes were stored in a transparent plastic bottle with a gauze lid filled with 10% sugar water in cotton as a source of feed (Kessler et al., 2013). The bottles containing the caught mosquitoes were then stored in plastic containers box with banana tree fronds attached to the inside of the walls to keep the air moist during the trip to the laboratory (Mattew, 2020; Suwito et al., 2010).

The determination of the caught mosquito species was carried out through several stages. The process of mosquito anesthesia in each sample bottle was by drop-

Figure 1
Time distribution matrix of mosquitoes' activities



ping 2 mL of chloroform solution (Sumanto, 2014) on a cotton swab of sugar water in the sample bottle and then closing the bottle tightly. After the mosquitoes passed out, they were identified microscopically. The acupuncture needle soaked with nail polish liquid at the end was then attached to the mosquito thorax. Then, the base of the needle was attached along with the cork media to make it easier to set the mosquitoes under the microscope lens. Morphological observations using a stereomicroscope were started from the head, thorax, body, wing, and legs of the mosquitoes. The identification refers to a mosquito identification key from The Walter Reed Biosystematics Unit (WRBU) of the United States Department of Defense via the online application at http://www.wrbu.org/VecID_MQ.html. Species identification was conducted at the Laboratory of Epidemiology of the Faculty of Public Health of Universitas Muhammadiyah Semarang.

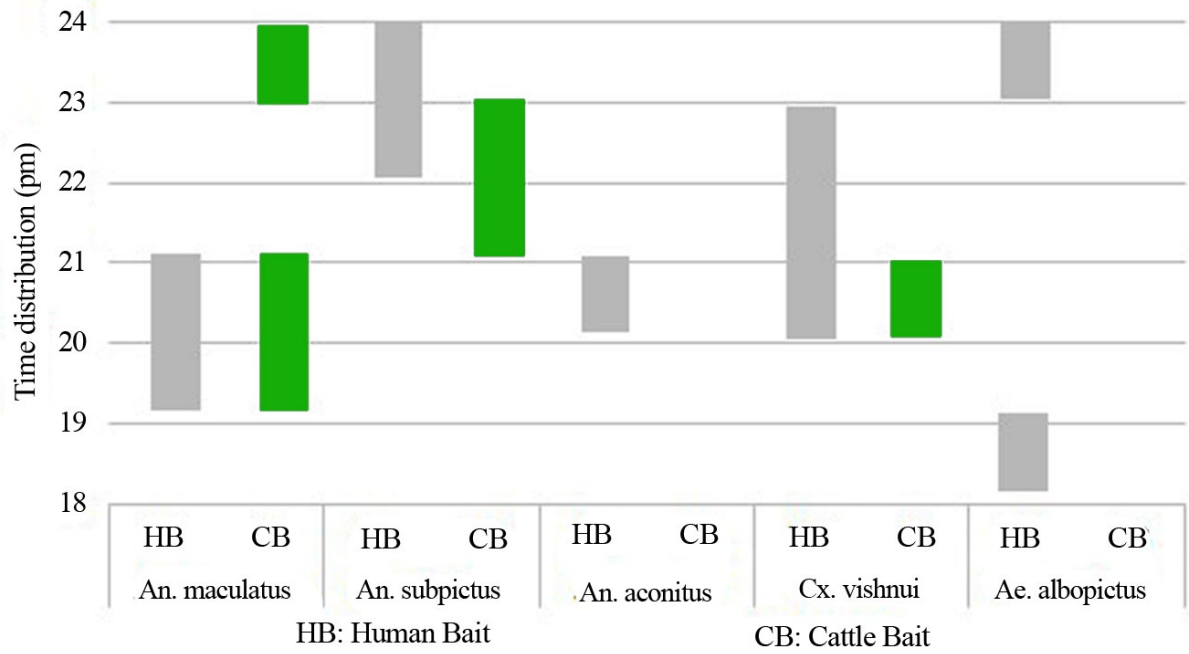
The data were first classified, and the proportion was further calculated based on the types of species found, the place of catching, and the type of blood bait preferred. Furthermore, cross-tabulation was carried out between the observed variables. The trend that occurred quantitatively was afterward narrated descriptively.

This study was approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Muhammadiyah Semarang with certificate of ethical acceptance number 029/EC/FK/2020

RESULTS

Mosquito catch had been carried out during the rainy season in October. Mosquitoes that were caught were active from 6.00 pm to 24.00 am. This result provided a unique fact because after 24.00 am, there was no single mosquito was caught, either by human bait people or livestock. Anthrophopilic mosquitoes were reported to have

Figure 2
The caught proportion of Anopheles species



started their activities earlier at 6.00 pm, while the zoophilic mosquitoes started appearing at 7.00 pm. In general, Anopheles was active between 7.00 pm - 24.00 am, Culex between 8.00 pm - 11.00 pm, and Aedes from 6.00 pm - 7.00 pm and 11.00 pm - 24.00 am. (Figure 1)

The three Anopheles species were caught at various hours. *An. maculatus* was caught between 7.00 pm - 9.00 pm either with human and cattle bait and reappeared at midnight at 11.00 pm - 24.00 am. *An. subpictus* was caught late between 9.00 pm - 11.00 pm with cattle bait and 10.00 pm - 24.00 am with human bait. *An. aconitus* was only caught between 8.00 pm - 9.00 pm by human bait. *Cx. vishnui* was caught by human bait from 8.00 pm - 9.00 pm, while those using livestock bait had a longer duration of time between 8.00 pm - 11.00 pm. *Ae. albopictus* was only caught by human bait at dusk from 6.00 pm - 7.00 pm and before dawn at 11.00 pm - 24.00 am. (Figure 1)

The mosquitoes that were found had two characteristics based on their blood feed preferences, namely the anthropophilic type, which sucked human blood, and the zoo-anthropophilic type, which sucked human blood as well as livestock, as seen in the (Table 1). The blood feed preferences of captured mosquitoes also provided information on the location of their activity. Zoophilic mosquitoes that preferred animal blood feed were caught at the location of the cattle sheds, while the anthropophilic species that preferred human blood feed were caught outside residential houses. *An. maculatus*, *An. subpictus* and *Cx. vishnui* activities were outside human houses and in cattle sheds, while *An. aconitus* and *Ae. albopictus* were relatively not found in the cattle shed area. An aconitus found in the field turned out to

Table 1
Blood Feed Preferences of The Mosquito

Species	Prefers of blood meal	
	Zoophilic	Antrophophilic
Anopheles		
An. maculatus	Yes	Yes
An. subpictus	Yes	Yes
An. aconitus	No	Yes
Culex		
Cx. vishnui	Yes	Yes
Aedes		
Ae. albopictus	No	Yes

prefer human blood food to livestock blood, while the other two Anopheles species preferred both types of blood feed. *Cx. vishnui* likes both types of blood feed and *Ae. albopictus* only likes human blood feed. *An. maculatus* dominated the catch (40.91%) followed by *Cx. vishnui* (27.27%), and the least was *Ae. albopictus* (9.09%). (as given in table 2)

The mosquito group that needed special attention was Anopheles because the study location was in the pre-elimination status of malaria. The proportion of Anopheles caught was different for each species. Calculated based on the existence of the Anopheles group only, *An. maculatus* was the species that dominated the catch (64.29%), followed by *An. subpictus* (28.57%) and *An. aconitus* (7.14%). (Figure 2)

Anopheles was mostly caught in cattle sheds (57.14%) than outside residential houses (42.86%). *An. maculatus*, which was considered the dominant species in Jatirejo village, was found more frequently in cattle sheds (42.86%) than outside residential houses. *An. subpictus* was found to be proportional between both locations (14.29%), while *An. aconitus* was not detected in cattle sheds. (Table 3) The main information from the results of this study was still found the Anopheles population as a malaria vector in the pre-elimination area of Purworejo regency and needs attention.

DISCUSSION

The reduction incidence of new cases in malaria-endemic areas in Purworejo re-

Table 2
The Dominance of Caught Mosquitoes

Species	Frequency (mosquito)	Percentage (%)
Anopheles		
An. maculatus	9	40,91
An. subpictus	4	18,18
An. aconitus	1	4,55
Culex		
Cx. vishnui	6	27,27
Aedes		
Ae. albopictus	2	9,09
Total	22	100

Table 3
The Location of Anopheles Catch

Species	Number of mosquitoes (tail/%)		Total
	Outside home	Cattle sheds	
An. maculatus	3 (21,43%)	6 (42,86%)	9 (64,29%)
An. subpictus	2 (14,29%)	2 (14,29%)	4 (28,57%)
An. aconitus	1 (7,14%)	0 (0,00%)	1 (7,14%)
Total	6 (42,86%)	8 (57,14%)	14 (100,00%)

gency has triggered some changes in local government policies toward eliminating this disease. New case data shows a change from indigenous cases to imported cases (Wibowo, 2020). This condition shows good progress towards massive malaria control.

In addition to handling malaria cases completely, to support this policy, serious vector control efforts are needed to prevent local transmission from such imported cases. This definitely requires complete information about the presence of malaria vectors (*Anopheles spp.)* in the region. This study provides important data and information for malaria vector control in pre-elimination areas.

The Anopheles, Culex, and Aedes mosquitoes that were caught at night provide interesting information. The dominance of Anopheles mosquitoes from catches at the site is a very logical finding (Shinta et al., 2013), considering that the previous catching location was a malaria-endemic area (Kuswantoro, 2015; Prabowo, 2019; Setyorini, 2016) and is currently in a pre-elimination status. Local malaria cases in this region have been successfully suppressed until 2018 (Bayu, 2018), although there are still import cases until 2020 (Wibowo, 2020).

This study found three important species of malaria vector mosquitoes with a high proportion. This condition showed that there was a high potential for local transmission of the malaria parasite, considering that imported cases were still found in this area. The three Anopheles mosquitoes have different habitats and bionomies. Anopheles was found in Jatirejo Village from 7.00 pm - 24.00 am and had a relatively shorter active duration. This is in line with the findings in Sukaraja Village, which reported the activity of *An. maculatus* was outside the home between 6.00 pm - 02.00 am (Ambarita et al., 2011). Two other species of Anopheles that were caught were *An. subpictus* and *An. aconitus*. *An. subpictus* types which were caught at 9.00 pm - 24.00 am, relatively late in the evening compared to *An. maculatus* activity. This finding was in line with reports from Iran, which suggested that the peak activity of *An. subpictus* was between 22.00 pm - 23.00 pm and 03.00 am - 04.00 am (Nejati et al., 2018) and in Rajabasa Lampung at 02.00 am - 03.00 am (Suwito et al., 2010). The catch of *An. aconitus* was

at 8.00 pm - 9.00 pm, which was in line with reports from Lampung, which found the highest density of this species was from 6.00 pm - 9.00 pm despite the activity throughout the night (Suwito et al., 2010).

Generally, the Anopheles population will be found in large numbers in malaria-endemic areas (Sinka et al., 2011), although it is not always the largest population (Kwansomboon et al., 2017). Most of the caught Anopheles are zoophilic. This is evident from the preference for Anopheles blood feed caught during the survey; relatively more livestock blood prefer. This result is consistent with previous reports where the most preferred livestock blood was goat blood (Nisrina et al., 2020). The proportion of the blood feed preference of this catch is in line with the area where it was caught. Zoophilic Anopheles was caught in livestock shed areas using goat blood feed, as previously reported (Munirah, 2021).

The three species of Anopheles also have different preferences for sucking blood. Even though *An. maculatus* and *An. subpictus* likes both human and animal blood, but *An. maculatus* is more zoophilic. This can be seen from the proportion of catches in the cage area, which is higher than around the residents' houses. Previous findings also reported *An. maculatus* feeds on human blood (Setiyaningsih et al., 2018) as well as livestock blood (Franco et al., 2014; Minirah, 2021), indicating that the species was zoo-anthropophilic. Meanwhile *An. aconitus* that was caught was the only anthropophilic. This result was in contrast with the findings in Thailand's Tak Province, which reported more findings of this species in livestock bait catches (Tananchai et al., 2019). The lack of aconitus findings in this location was very likely due to the unavailability of rice fields with stagnant water as the preferred breeding place.

An. maculatus was found to be the dominant species. This is in line with the conditions of the natural environment, which is the habitat of this species. Jatirejo Village is located in a hilly area with an elevation of 226 m asl (Susanto, 2019), with the geographical conditions of hilly land with dry fields and plantations without any rice fields (Wazzirudin, 2018). The location of village, located at the top of the hills, has the potential for finding springs in the plantation area and hilly forest. This spring is one of the habitats favored by *An. maculatus* (Ambarita et al., 2011). This finding is in line with the results of previous studies on malaria vector findings in endemic areas in Bukit Menoreh (Enny et al., 2007). Another area in Purworejo Regency, namely Polowangi Village, Pituruh Subdistrict, also reported the same findings regarding a maculatus dominance in the catch (Shinta et al., 2013), while in Sendangsari Village, Bener District, vectors of this species were also found (Setiyaningsih et al., 2018). Apart from Indonesia, *An. maculatus* group appeared to be one of the important malaria vectors in Southeast Asia, among which it has also been reported in Thailand and Laos (Marcombe et al., 2020; Tainchum et al., 2015).

Zoophilic properties of *An. maculatus*, which prefers animal blood feed over human blood, has been reported in various re-

gions (Munirah, 2021; Franco et al., 2014; Muchid et al., 2015; Setiyaningsih et al., 2018). The availability of animal blood feed sources is an important factor for zoophilic vector reproduction. Jatirejo Village is the center for the development and cultivation of Etawa crossbreed goats (Nisrina et al., 2020), so goat farming is widespread in this village area (Wazzirudin, 2017; Wazzirudin, 2018; Susanto, 2019). This has the potential to be one of the factors supporting the acceleration of the gonotrophic cycle in the zoophilic Anopheles breeding (Laurent et al., 2016).

On the other hand, the environmental conditions of this village are also suitable for mosquitoes, such as subpictus. This species likes the holes and excavated soil filled with rainwater (Noshirma & Adnyana, 2016). The existence of a large livestock population has the potential to cause the emergence of holes used for cattle footprints. Excavation of former fish ponds that are not reused has also become a preferred habitat (Noshirma & Adnyana, 2016; Setiyaningsih et al., 2018). *An. aconitus* is the species that is least found in this area, considering that this species likes the habitat of rice fields with water running in trenches. This situation was not found in villages located in this hilly area (Susanto, 2019).

CONCLUSIONS

Three malaria vector species were still found in the malaria pre-elimination area in Purworejo Regency, namely *An. maculatus*, *An. subpictus*, and *An. aconitus* respectively. This condition indicated that the potential for local malaria transmission is still there, with different levels of dominance according to habitats and blood feed preferences. This situation requires planning appropriate vector control measures, both based on habitat and bionomics. Domination of *An. maculatus* in the population of Etawa crossbreed goats should be a concern because this species is zoophilic, so the blood feed supply is adequate in the presence of goats. It is highly recommended to further research Anopheles populations in malaria-endemic areas before and after the vector control program was implemented.

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