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The Risk of Mosquito-borne Diseases Related to Mosquito Fauna Richness and Livestock Placements in South and West Sulawesi, Indonesia

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Abstract

BACKGROUND: The local fauna of mosquitoes may have an essential role in the transmission of mosquito-borne pathogens.

AIM: The future risk of mosquito-borne diseases needs to be considered by the presence of factors that support mosquitoes and pathogens, such as the habitats, presence of host reservoirs, and placement of livestock in settlements.

METHODS: Mosquito catching methods used Animal Barrier Screen (ABS), Kelambu Trap (KT), and Human Landing Catch (HLC) in the wet and dry season. The role of a large animal in getting mosquito bites was analyzed based on the proportion of mosquitoes sampled by HLC to all collected mosquitoes. The potential vector of mosquitoes was projected based on the habitat, species density, and presence of host reservoirs.

RESULTS: Pasangkayu district had more mosquito fauna compared to North Toraja and Maros. However, the separated placement of livestock in North Toraja resulted in fewer mosquito bites to humans compare with Maros, where livestock was caged or tied directly beside individual houses. The separated placement of livestock in North Toraja and Pasangkayu acted as a barrier, while scattered placement among houses at Maros acted more as a mosquito attractant.

CONCLUSION: The habit of placing livestock separate from human settlements may reduce mosquito bites, reducing the risk of contracting mosquito-borne diseases. This finding proves using livestock as an outdoor vector control strategy to protect mosquito bites and disease transmission.

Introduction

The bloodsucking behavior of mosquitoes allows them to acquire pathogens from one host and bring it to others [1], so transmit the diseases caused by the pathogens. Disease transmission involves cycles between parasites, hosts, vectors, and a suitable environment. The selection of a suitable vector depends on the competence of each specific species. Mosquito-borne diseases that spread worldwide have a severe impact on human health, social, and economic in parasitic diseases such as malaria and lymphatic filariasis, or viral diseases such as Japanese encephalitis, dengue, chikungunya, and yellow fevers [2], [3], [4].

The parasite that causes malaria in humans (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*) is solely transmitted by *Anopheline mosquitoes* [1], [5]. More than 400 species of *Anopheles* worldwide and about 40 species play a role in transmitting those *Plasmodium* parasites. Another debilitating parasitic disease with severe social and economic impact is lymphatic filariasis caused by nematodes such as *Wuchereria bancrofti*, *Brugia malay*i, and *Brugia timori* [1], [3]. The mosquito vectors of the filarial worms include the genera of *Aedes*, *Ochlerotatus*, *Anopheles*, and *Culex* [6]. In addition, mosquito-borne arboviruses are transmitted by several mosquito genera, including *Anopheles*, *Aedes*, and *Culex* [7], [8] mosquitoes which cause viral diseases in humans mainly from three families: Togaviridae (e.g., Chikungunya, Ross River, Eastern Equine Encephalomyelitis, Western Equine Encephalomyelitis, Venezuelan Equine

Encephalomyelitis, O’Nyong-Nyong, The Sindbis Virus Complex, and Semliki Forest Complex), Flaviviridae (e.g., Yellow fever, Dengue, West Nile), and Bunyaviridae (e.g., The California serogroup, Bunyamwera complex, and Turlock group) [1].

Sulawesi Island is a unique island in Indonesia since it was made up of different geological plates of the earth’s crusts [9]. It is the mainland within the Wallace region, a transitional region bridging the zoogeographic of Asia and Australia. The animals are unique as they mixed the Asian and Australian origin characters [10]. The ecology of Sulawesi varies from coastal ecology, swampy forest to the deep jungle in mountains area [9]. The type of mosquito habitats also varies following its ecological landscape, affecting the mosquito fauna of different areas. Human inhabitants of the Sulawesi Island also vary in cultures and habits, including how they keep animals as livestock [11], [12]. Most of the large mammals in Sulawesi are still domesticated in traditional ways inherited from their ancestors. There are different ways people keep animals related to their house position. Most people in traditional villages keep their animals just under or behind their high wooden houses, so the animal placements are scattered among humans. In other places, people keep their large animals concentrated in a particular area that separated them from the housing area [11].

We conducted a mosquito survey in several different ecological types and cultures and saw whether the observed parameters might increase the risk of contracting mosquito-borne diseases. We observed the type habitats, mosquito abundance, genus, and species richness, and people habits of placing large mammals, such as cows, buffaloes, and pigs, related to their housing position, the risk they may face to get bitten by mosquitoes that are known to transmit disease.

Materials and Methods

Study area

The study was conducted from October 2018 to September 2019 in three districts of Sulawesi Island: Maros and North Toraja Districts in South Sulawesi Province and Pasangkayu District in West Sulawesi Province (Figure 1). The three sites were once part of South Sulawesi Province and traditionally were regarded as the same geographical area despite differences in its dominant local tribe communities. The three districts represent different ecological types, that is, lowland, highland, and coastal ecosystems.

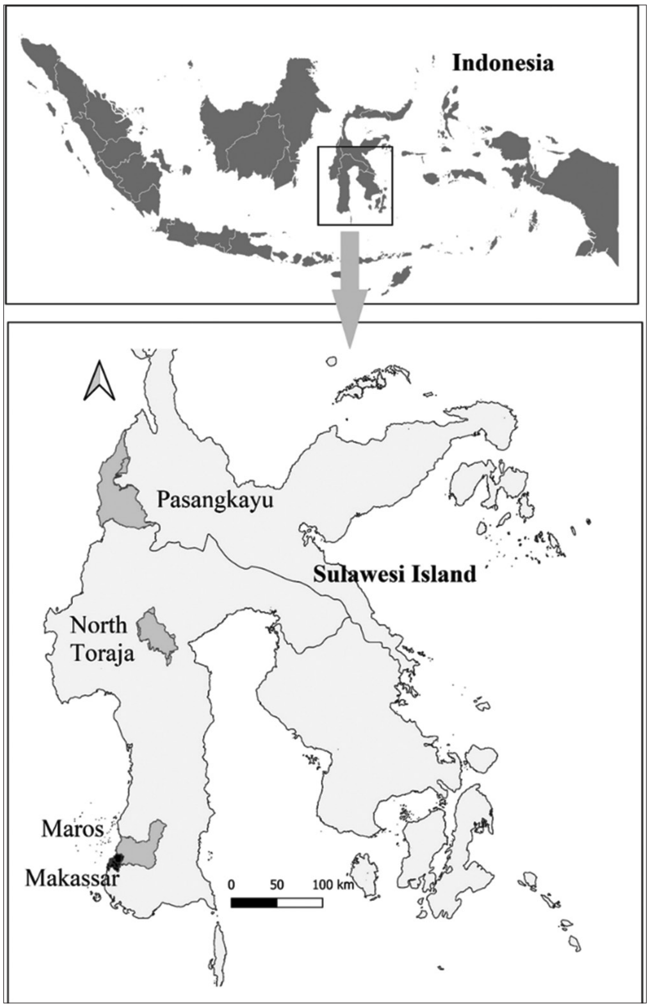

Figure 1: Study sites at South and West Sulawesi

Maros

Maros is a lowland district bordered by Makassar, the capital of South Sulawesi Province, with the district’s capital is an urban area about 30 km from the center of Makassar city. Its 353,121 population [13] within 1619.2 km² was mostly Makassar and Bugis tribes, the dominant tribe in South Sulawesi. Most ecological types are karsts, rice fields, and lowland forest, with some brackish fishponds situated at its short coastal line. Most of its population were farmers, with some fishermen in the coastal area. The National Park of Batimurung-Bulusaraung is situated within this district where the endemic monkey species *Macaca maura*, which may harbor some non-human primate malarial parasites, is protected. The mosquito sampling area was in Arra village of Tompobulu subdistrict, a settlement surrounded by rice fields and lowland forests.

North Toraja

The highland of North Toraja, situated 704–1646 m above sea level, distanced 336 km from Makassar city with 1151.5 km² of the mountainous area consist of primarily highland forests scattered with highland rice field, coffee, and cocoa plantation and human settlements. Its 247,157 population [14] were mostly Toraja tribe with few other immigrant tribes. This area has a unique culture in its burial ceremony since it would include hundreds of animal sacrifices, particularly buffaloes and swine, which might amplify hosts for some arboviruses such as the Japanese encephalitis virus. Imported malaria cases were not uncommon in this area since many of Toraja peoples work in the malaria-endemic area of Papua and Borneo. The mosquito sampling area was in a highland village surrounded by rice fields and forested areas.

Pasangkayu

Pasangkayu is relatively a new district after being separated from Mamuju District of West Sulawesi with a 3044 km² administrative area. Situated about 700 km from Makassar city with 242,417 population [15] mostly immigrant from other parts of Sulawesi Island and other Indonesia regions. It is a mixture of tribes from South Sulawesi, West Sulawesi, Central Sulawesi, and other tribes from other islands, mainly from Jawa and Bali. A mixture of Buginese, Mandarese, Javanese, Balinese, and local isolated tribe Kaili Da'a of Central Sulawesi. Most of the people work as farmers for palm, cocoa, and rice plantation. The ecological types were mostly coastal with brackish fishpond, swampy, and lowland forested areas. This area is rich with swallow bird populations since swallow bird-nest cultivation is becoming a profitable business. Mosquito collections were performed in a hilly forested area within the range of settlement area of the semi-nomadic tribe of Kaili Da'a. Pasangkayu once was an endemic malaria area in Sulawesi and recently decreased its cases following the National Campaign for Malaria Elimination initiated in 2009. However, there were still sporadic cases of indigenous malaria.

Mosquito collection

Mosquito was collected twice for each site to represent wet (Oct-Dec 2018) and dry (Jul-Sep 2019) seasons. Mosquito was collected by ABS and KT methods [16], [17], [18], as well as outdoor HLC, which is the WHO's standard method for malaria vectors collection [19]. Mosquito collections were performed using aspirators to catch mosquitoes that landed on the surface of the ABS, KT, and human body and stored in paper cups hourly from 6 pm to 12 for ABS and KT and from 6 pm to 6 am for HLC. The collection day for each trap was set for 10 days, but some traps were less than 10 days because of trap failure due to extreme weather.

The collected mosquitoes were killed by cotton-chloroform put on the cover-net of the paper cup and identified under a dissecting stereomicroscope (Nikon SMZ745, Japan) following the Indonesian's Identification Keys of Female Mosquitoes by The Ministry of Health [20]; for *Aedes*, *Culex*, and *Mansonia* [21].

Livestock placements and trap positioning

Three types of traps used in this study have different targets: ABS target mosquitoes attracted and come around large animals and installed near or facing animal cages of place where people tied their pigs, cows, or buffaloes; KT was purposed to catch hovering mosquitoes and placed in an open space were flying mosquitoes may land and rest in the trap; while HLC was purposed to catch mosquitoes that attracted to human and come to land on human bodies of the collectors. Traps were set following how differently each site shows how animal cage position relative to houses position in a human settlement may affect the frequency of mosquitoes to come and bite humans.

In Toraja, pig and buffalo cages were orderly placed in one line at the edge of the village, separated into a group of houses and situated between houses and habitats. In Pasangkayu, even though cows were tied separated from houses but not orderly placed in one line, while in Maros, the animal cages were just under or beside people's houses, so the animals scattered between houses without any clear separation to the houses. Positioning of traps followed the place of animals for the ABS, open space for KT, and house position for HLC (Figure 2).

Mapping

Study site and mosquito habitat location were marked using GPS Garmin Montana 680 and processed using the GIS Software of Quantum GIS ver. 3.12.3.

Ethics

This research did approve by the Health Research Ethics committee of the Hasanuddin Medical Faculty with the attached number 710/UN4.6.4.5.31/PP36/2020.

Data analysis

Data will be presented in tables and diagrams. Human Biting Rates (HBR) is the prevalence of mosquitoes that came to bite humans (number collected by HLC) per 1000 mosquitoes from the total collected mosquitoes by all three methods used. Data processing used Microsoft Excel.

Results

Mosquito richness

Total 17,507 female mosquitoes were collected from the three sites: Maros (6034), North Toraja (6370),

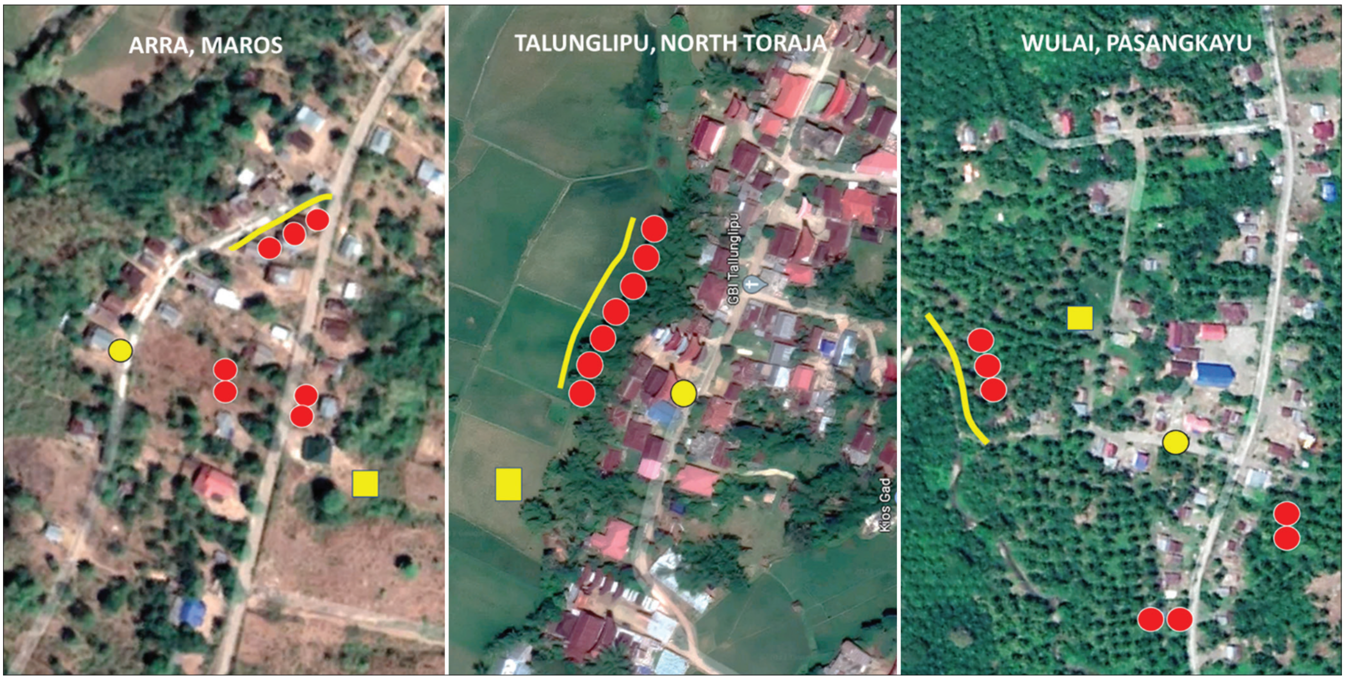

Figure 2: Trap positioning and placement of livestock in villages of Arra (Maros District), Talunglipu (North Toraja District), Wulai (Pasangkayu District). Red circles: animal (pig, cow, buffalo) placement, yellow circle: HLC position, Yellow square: KT position, Yellow line: ABS position

and Pasangkayu (5103) consisted of 45 species from nine genera: *Aedes*, *Anopheles*, *Armigeres*, *Culex*, *Coqulittideae*, *Lutzia*, *Mansonia*, *Mimomya,* and *Uranotaenia*. The dominant genera were more or less similar to other places: *Aedes*, *Anopheles*, and *Culex* were the main mosquitoes collected, 9.1% (2.3–20.8%), 21.5% (8–32.4%), and 68.7% (61–74.3%), respectively. *Culex* invariably was the most common mosquito found in the three study sites (Figure 3).

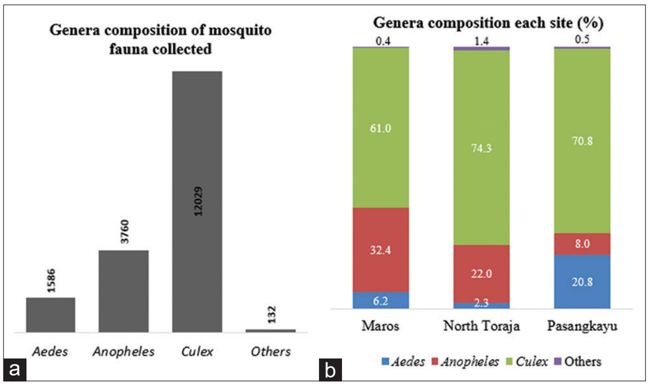

Figure 3: The number of mosquitoes collected for main genera (a) and its composition in the three study sites (b)

Of the 45 species collected from three locations, seven species were collected only in Pasangkayu, four species in North Toraja, and three species in Maros. Very few numbers of human dwellings associated with *Aedes* were found. *Aedes albopictus* were found in all three sites, whereas *Aedes aegypti* was only found in Maros. However, there was a significant number of *Aedes vexans* found, and one unidentified species of *Aedes*. In addition, there were 14 species of Anopheline collected: *Anopheline argyropus*, *Anopheline barbirostris*, *Anopheline barbumbrosus*, *Anopheline flavirostris*, *Anopheline kochi*, *Anopheline maculatus*, *Anopheline minimus*, *Anopheline nigerrimus*, *Anopheline peditaeniatus*, *Anopheline subpictus*, *Anopheline sulawesi*, *Anopheline sundaicus*, *Anopheline tessellatus,* and *Anopheline vagus.* *Culex* found in three locations consisted of 14 species, that is, *Culex bitaenhyorinchus*, *Culex fuscocephalus*, *Culex gellidus*, *Culex hutchinsoni*, *Culex infula*, *Culex longicornis*, *Culex malayi*, *Culex minimus*, *Culex nigropunctatus*, *Culex sitiens*, *Culex quinqifasciatus*, *Culex tritaenhyorinchus*, *Culex vishnui*, and *Culex whitmorei.* Mosquitoes from other genera (*Armigeres*, *Coqulittideae*, *Lutzia*, *Mimomya*, *Mansonia*, and *Uranotaenia*) contain only one or two species (Supplementary data 1).

The richness of mosquito fauna at each site was reflected by their Community Composition Measure (CCM) values, which show the individual number of mosquitoes, number of species, and number of genera(22). For example, the CCM values for Maros, North Toraja, and Pasangkayu were 6034(27) [5], 6370(30) [6], and 5103(34) [8], respectively (Table 1).

Table 1: CCM value as a function of fauna richness at each site in the wet and dry season

Season collected	Trap night	No. Mosquito	No. Species	No. Genera	CCM
Maros		6034	27	5	6034(27) [5]
Wet	30	3228	15	5	3228(15) [5]
Dry	30	2806	23	5	2806(23)[5]
North Toraja		6370	30	6	6370(30) [6]
Wet	30	2628	26	5	2628(26) [5]
Dry	26	3742	24	4	3742(24) [4]
Pasangkayu		5103	36	8	5103(34) [8]
Wet	28	4133	19	5	4133(19) [5]
Dry	24	970	27	7	970(27) [7]

CCM: Community Composition Measure.

Bloodsucking activity and human biting rate

Mosquitoes that come to bite humans (collected by the HLC method) were far lower than those attracted to feed blood on animals (collected by the ABS method) or the swarming mosquito in an open space (collected by the KT method), with Maros show relative higher number of human-attracted mosquitoes compare to both North Toraja and Pasangkayu. Furthermore, the number of mosquitoes collected from non-human traps was higher in the early night (6–7 pm) and decreased toward midnight, except for Pasangkayu, where the peak was around 9–12 pm, while mosquitoes that came for human blood show more stable pattern from early to midnight. The graph in Figure 4 confirmed the higher human-blood sucking activities of mosquitoes in North Toraja compared to the other two sites.

Of 17510 mosquitoes collected, 568 were collected by the HLC method when they come to bite and landed on the human bodies. Despite the comparable number of mosquitoes collected at all three sites, the Human Biting Rate (HBR) varied significantly, with Maros had the highest HBR (77.5), followed by Pasangkayu (13.5) and North Toraja had the lowest one (5.0). The lowest HBR for North Toraja was also true for *Anopheles* and *Culex* mosquitoes (*Culex* HBR 121.1, 5.6, 3.0 and *Anopheles* HBR 9.7, 105.5, 0.7 for Maros, Pasangkayu and North Toraja, respectively), while Pasangkayu had the highest Anopheles HBR (105,4) with Maros had the lowest one (0.7). The opposite occurred for the genus *Aedes*, where North Toraja had the highest HBR (73.8), with Maros and Pasangkayu had much lower (5.3 and 3.8, respectively).

It was also interesting to see differences in prominent mosquito genera collected by HLC, as shown in Figures 5 and 6. *Anopheles* was the dominant mosquitoes collected by HLC in Pasangkayu, while in North Toraja and Maros, *Aedes* and *Culex* were the dominant genera associated with HLC, respectively.

Mosquito born-pathogen potential vector

Of 45 mosquito species collected, 31 have been reported associated with either parasites or arboviruses (Supplementary data 2). Species of *Aedes* related to arboviruses (six species) and filarial worms (two species); *Anopheles* were associated with human *Plasmodium* (ten species), arboviruses (five species) and filarial worms (six species); while *Culex* associated with avian *Plasmodium* (three species), arbovirus (eight species), and filarial worm (two species). In addition, other genus (*Amigeres*, *Lutzia*, *Mansonia*, and *Uranotaenia*) were related to avian *Plasmodium* (three species), arboviruses (three species), and filarial worm (two species) (Supplementary data 2).

Discussion

The diversity of mosquito fauna of Sulawesi Island is high in terms of genera number. Of 44 known mosquito genera worldwide [23], 20.5% (9 species) were found on the island. *Culex* invariably was the most common mosquito found in the three study sites, followed by *Anopheles* and *Aedes* as the second and third predominant mosquitoes. The other six genera contributed only 0.8% of the total mosquito collected. This composition was not uncommon in other places [24], [25]. Predominant mosquitoes found in each site were related to the sites' most common habitats and characteristics.

The most frequent species of genus *Culex* collected in this study was the species associated with paddy fields, that is, *Culex tritaeniorhynchus* that distributed evenly in the three sites, as all sites have paddy fields, with the Pasangkayu having the least proportion and the least number of this species. The urban and polluted-water habitat mosquitoes, the *Culex*

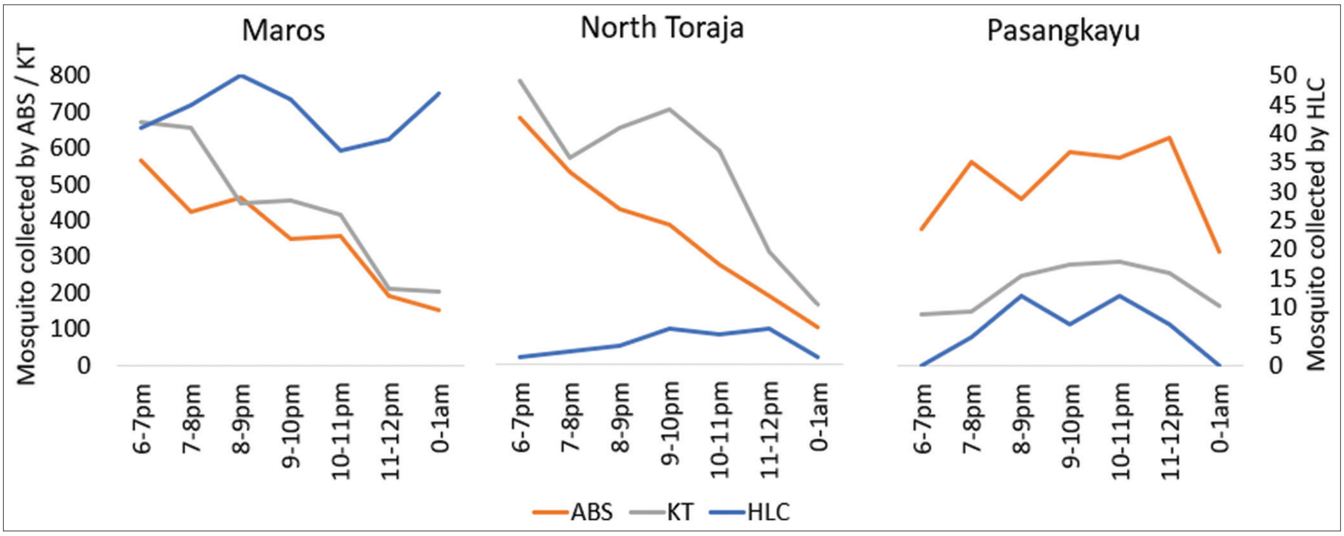

Figure 4: Differences in a mosquito collected using human-associated trap (HLC) and non-human methods (ABS and KT)

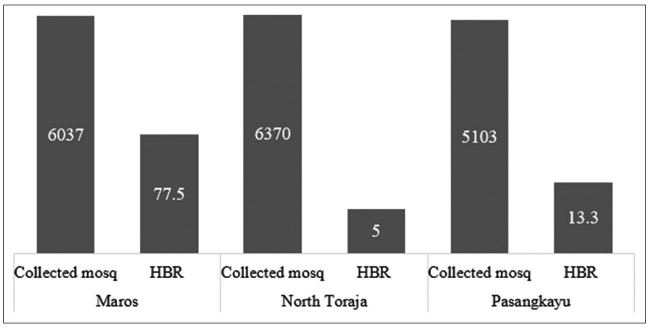

Figure 5: The proportion of mosquitoes that come to bite humans per 1000 mosquitoes collected (HBR, Human Bite Rate) in each site. Maros show the highest HBR, while North Toraja had the lowest

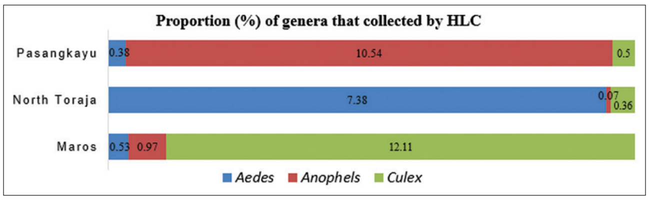

Figure 6: Percentage of mosquito genera collected by HLC in the three study sites

quinquefasciatus was significantly higher in Maros than the other two districts since this site was the direct neighbor of the highly urban city of Makassar with the most pollutant in its water habitats. It is also true for *Culex vishnui* that breed in open ground pools with direct sunlight, such as grassy pools and paddy fields. This species is found more in Pasangkayu that has more grassy pools.

Anopheles species were more prevalent in Maros and North Toraja. *Anopheles barbirostris* and *Anopheles kochi* were the dominant species in North Toraja with more forest and swampy area [26]. *Anopheles vagus* was the dominant species in Maros with most rice fields, as well as *Anopheles minimus* and *Anopheles maculatus* that were associated with shaded streams, which found more in Maros. The brackish pool- associated *Anopheles sundaicus* was only found in the coastal area of Pasangkayu (Supplementary data 3).

Aedes aegypti and *Aedes albopictus*, well-known vectors for dengue viruses, were very few collected. Only one individual of *Aedes aegypti* was collected in Maros, the nearest site to the highly urban city of Makassar. *Aedes aegypti* is usually more prevalent in highly urbanized areas, while *Aedes albopictus* in rural, suburban, and vegetated urban areas. *Aedes albopictus*, known as the garden mosquito [27], was also found in the more suburban and rural places of North Toraja and Pasangkayu, although in small numbers.

The scarce of these daytime-biter mosquitoes may relate to the trapping time that is performed at night. Most *Aedes* species collected in this study were *Aedes vexan* and *Aedes vigilax*, which both breed in pools and swampy areas. In comparison, *Aedes vexans* commonly found in all three sites, the coastal-related *Aedes vigilax* (supplementary data 2) was more prevalent in Pasangkayu with predominant brackish fishponds, salt marshes, and temporary ground pools.

The species richness reflected by its CCM value shows that despite its lowest number of mosquitoes collected, Pasangkayu district has the most genera (n = 8) with most species (n = 34) of mosquito fauna Maros and North Toraja has a comparable number of genera and species. The unique Pasangkayu ecosystem that combines coastal area and deep primary forest not so distantly apart provides them plenty of unique habitat-specific species. There were seven species found only in Pasangkayu that related to a specific type of habitat. At least *Aedes butleri*, *Aedes dux*, *Coquillettidia crassiper*, *Mi. aurea*, and *Uranotaenia* sp. were mosquitoes associated with brackish habitat or a coastal area, while *Aedes flavipennis* were related to the forest ecosystem. Differences in the CCM value of wet and dry seasons reflected how habitats differ and change between seasons. Pasangkayu, with the vast coastal area and open river edges, provide more temporary pools that are both affected by flood in the wet season and drought in the dry season, and this situation was well reflected by differences CCM in wet, 4133(19) [5], and dry season, 970(27)[7]. It appeared that number of genera and species was richer in the dry than the wet season. This change is characteristic of coastal areas with lagoons, estuaries, and big rivers with a large area of riverbeds since the stagnant water fill most water bodies in the dry season will be flushed out by a flood in the wet season, and hence this type of ecology will have more mosquitoes and mosquito-borne diseases in the dry season rather than the wet season. As Maros too has a coastal area, though, in less degree than Pasangkayu, it showed a pattern of diversity change more or less similar to Pasangkayu, where dry season increases the diversity of mosquito fauna. Inversely, in more forest and swampy areas, paddy and grassy fields, like in North Toraja, rain-filled water ground pools and water bodies increase in the wet season and lack of water in the dry season, so less mosquito and associated diseases in the dry season.

Activity time of mosquito in Maros and North Toraja shows an early night biting trend that decreases toward midnight, while in Pasangkayu show a relative stable curve that initiated few hours after sunrise. The mosquito trapped using the animal attractant (ABS) and the free flying-targeted trap (KT) show a similar trend, but human-related activity reflected by mosquitoes trapped by HLC shows a different pattern. The human biting activities seemed to begin at 6 pm and increase from 8 pm to 10 pm. However, the number of mosquitoes collected by human-associated trap (HLC) was far less than those by non-human-associated trap (ABS and KT). These results were similar in pattern with previous study [28], but show a different trend in other studies [29].

The active biting time of mosquitoes influences their chance to be a vector of diseases related to local human habits and their night activities, as well as the use of insecticides. It seemed that mosquitoes that come to bite humans increase toward midnight when most people go to sleep. The evolution of these human-biting habits is probably increasing the level of success to get blood and lower the mortality risk during blood-fed on sleeping host [30]. The other story may differ in places where extensive insecticide-impregnated net applied, such as LLINs (long-lasting insecticide nets in highly malaria-affected areas such as in severe burden of malaria of African countries). The use of LLINs for sleep at night may give selective pressure to mosquitoes toward the individual variation that tends to bite at early night so escaping the death chance of interfering with the LLINs used toward midnight as people going to sleep. The consistent habit of sleeping under LLINs may switch the dominant traits of mosquito vapor early night blood-fed. It was documented in some malaria-endemic countries that treated with LLINs to the lower malaria [31] by preventing mosquito biting, increase mortality and reduce density.

It is interesting to note the data of human-biting prevalence in the three study sites, denoted here as the human biting rate per 1000 mosquitoes collected (HBR). As study sites differ in people's habits of keeping their livestock animals related to their houses, we observed that the HBR of Maros was significantly higher than the other site. The range of total mosquito number per hour in Maros was about 40–50, while in other sites ranged from 0 to 10 only. Maros people differ from Pasangkayu and North Toraja in the placement of their large mammals. Different from North Toraja and Pasangkayu people who keep their animals at a particular position separated from their houses, people of Arra village in Maros were keeping their livestock animals just directly behind, besides, or under their high wooden houses that were characteristic house style of Buginese and Makassarese tribes which predominant in Maros. It resulted in a scattered animal placement among people's houses. In contrast, the people of Tallunglipu village of North Toraja concentrated their large animals such as pigs and buffaloes along the border of their village and not directly mixed within the housing area. Habits of people in Wulai village of Pasangkayu was somewhat a transition style of Maros and North Toraja in which they placed their animals not directly within housing area but separated in a different location at the outer side of the housing area as in Figure 2. Interestingly, the HBR of the three villages was associated with the separation (or mixture) of human-animal position within a village. Maros with mixed human-animal position had the highest HBR, 77.5/1000 mosquitoes collected, while North Toraja, on the other hand with a concentrated and separated animal from the human position, had the lowest HBR, 5/1000 mosquitoes. Pasangkayu was somewhat in between the other two sites with HBR 13.3/1000 mosquitoes.

Considering the chance to contract diseases from mosquito bites, the arrangement of human-animal position within a village may play an essential role in mosquito-borne transmission. The use of animal barriers for protection from disease transmission was used in malaria prevention [32], [33], [34]. The separation of animals from humans for certain distances may attract mosquitoes to feed and be full within the animal range and not necessarily looking for other human hosts for blood meals. The pungent smell of large animals and its secretion might be important attractant factors to direct the mosquito toward its position and ignore the human host's presence. This observation may open a possibility for using concentrated large animals positioned at a certain distance around human settlements as a trap to reduce mosquito vector density and decrease the transmission of mosquito-borne pathogens. The combination of animal position with the lethal trap placed near or around animals may act as outdoor protection from essential diseases such as malaria. Placing an insecticide-impregnating net around large pigs or buffalo cages might be used as a lethal trap for mosquitoes. It can increase the mortality rate of mosquitoes in that area, lower the density, and swift the mosquito population to younger age that cannot transmit specific pathogens that need one to 2 weeks incubation period in mosquitoes, hence, can be used as outdoor protection for mosquito-borne disease transmission. The application of certain parasitic drugs, such as ivermectin, in these large mammals [35], [36] kept as livestock may also act as live lethal traps for mosquitoes and control disease transmission.

From the previous result, there are three pool genus positive from Maros: one *Culex* pool positive for flavivirus, one *Anopheles* pool positive for Alphavirus, and one *Armigeres* pool positive for flavivirus [29]. The chance of mosquitoes being a vector for specific pathogens may be related to their preference for human hosts rather than the animal. The more certain species bite humans, the more likely they can be a transmission vector for disease. JE antigen was found in *Anopheles peditaeniatus* [37], this mosquito was found in a minimal amount in North Toraja (n = 2) and Pasangkayu (n = 2).

Mosquitoes from the genus *Aedes* generally occupy containers in and around settlements, especially *Aedes aegypti* and *Aedes albopictus*, which is the primary vector of many arboviruses (dengue, chikungunya, zika, and yellow fever) [8], [38]. *Aedes aegypti* in Australia has been shown to be a competent zika vector, especially with its anthropophilic behavior [39]. Besides that, *Aedes aegipty* has flavivirus insect-specific, that is, Cell Fusing Agent virus (CFAV) found in the USA, while *Aedes albopictus* has Aedes flavivirus (AeFV) in Japan and Xishuangbanna Virus (XFV) in China [40]. Unlike the house mosquitoes, *Aedes vexan* tend to have breeding sites far from settlements. These mosquitoes choose to breed in much water, residual floods, or the edge of a pond shaded by trees. The existence of water sources such as rivers and ponds in the three locations means that these mosquitoes can be easily found. *Aedes vexan* can transmit West Nile (WN), Wesselsbron [41] and Potosi virus in South Caroline. In addition, it also has insect-specific flavivirus, that is, Chaoyang Virus (CHAOV), found in China [40]. Unlike the previously mentioned, *Aedes vigilax* prefer in saline and muddy habitats (Knight *et al*. 2012). This condition is very suitable for Pasangkayu, which is surrounded by swamps and mangrove forests so that these mosquitoes are abundant in this place. *Aedes vigilax* transmitted Murray Valley Encephalitis virus and Zika virus (with a prototype African strain) in Australia [39]. In addition, *Ae. vigilax* also transmits Liao Ning virus (LNV), Ross River virus (RRV), Salt Ash virus (SASHV), and Edge Hill virus (EHV) in Australia [42].

Anopheles, which is known as a malaria vector, is found mostly in Maros. However, the main vector of *Plasmodium*, *Anopheles barbirostris,* is very abundant in North Toraja compared with Maros and Pasangkayu. Others mosquito vectors such as *Anopheles barbumbrosus*, *Anopheles nigerrimus* and *Anopheles vagus* is also abundant in this area; this is due to the presence of breeding mosquitoes such as rice fields and livestock (such as pigs, cows, and buffaloes) as *Plasmodium* animal reservoirs. This potential malaria vector is strongly supported by the North Toraja people who work in Papua, known as endemic malaria areas.

Anopheles are also found in Maros, and this is supported by rice fields, gardens, and rivers (which are also similar to North Toraja, even though the Maros people live permanently in the area by working as farmers or animal breeders. Large numbers of *Anopheles* species are found in Pasangkayu but less abundant than North Toraja and Maros. The existence of ecosystems such as swamps seems to support the development of this type of mosquito.

The spread of malaria in 2016–2018 shows that cases in North Toraja were higher than in Maros and Pasangkayu (supplementary data 4). It shows that the existence of North Torajans expatriates is very influential in contributing to the incidence of malaria in Sulawesi. Malaria cases in Maros have decreased over the past 3 years, although the presence of very high *Anopheles* is a factor that has not been eliminated. The number of cases of Pasangkayu each year is small, and this is comparable with *Anopheles* which is less than Maros and North Toraja. Research on arbovirus in *Anopheles* is still rare, even though it has the potential to become an arbovirus vector because of its antrophilic nature and ability to harbor viruses. Its existence around humans as a malaria vector can be misdiagnosed due to malaria or fever caused by viruses or the phenomenon of human malaria and arbovirus coinfection [43]. Several viruses have been found in *Anopheles*, that is, Anopheles Minimus virus (AMIV) in *An. minimus*, viruses from the iridovirus genus can cause apoptotic responses in vertebrate and invertebrate cells [44]. Tibet Orbivirus (TIBOV) in *Anopheles maculatus* is from the orbivirus genus in Tibet, China, in pigsty rural. It is not yet known that it infects humans or animals [45].

Anopheles maculatus and *Anopheles minimus* are found in small numbers in Maros (n = 120 and n = 254, respectively) and Pasangkayu (n = 35 and n = 5, respectively). Kampung Karu Virus (KPKV) is an insect-specific flavivirus found in *Anopheles tessellatus* in Sarawak, Malaysia [40]. *Anopheles tessellatus* was found in all three locations in small numbers. In *Anopheles sundaicus*, Semiliki Forest Virus (SFV) was also found by infecting it via membrane feeding. This mosquito was only found in Pasangkayu (n = 147). *Anopheles* can also be a vector for lymphatic filariasis, including *Anopheles barbirostris*, *Anopheles flavirostris*, *Anopheles maculatus, Anopheles minimus, Anopheles subpictus,* and *Anopheles vagus. Anopheles maculatus* and *Anopheles minimus* is found in abundance in Maros. *Anopheles vagus*, which is the primary vector of malaria [46], was abundant at all three sites. Mosquitoes from others genera that can act as lymphatic filariasis vectors such as *Aedes aegypti, Aedes albopictus, Lz vorax., Lz. fuscana,* and *Uranotenia* were also present in small amounts at all three sites. Many factors are needed in disease transmission, including the host and source of the diseases. Fortunately, in Sulawesi, cases of lymphatic filariasis are very rarely found; in Toraja, during the last 5 years, there were only two cases and not at all in Maros, while in Pasangkayu, only one patient was reported (Supplementary data 4).

Mosquitoes of the genus *Culex* are a strategic vector for arbovirus transmission, especially JE. As the most abundant mosquito, *Culex tritaenhyorinchus* strongly supports JE transmission, especially in the northern Toraja region, which has many pigs. *Culex* is found in abundance in all three locations and mostly in North Toraja. The vast rice fields in Maros and North Toraja and the presence of rivers and gardens in the three locations are strategic breeding places for *Culex*. The mosquitoes that were abundant among all is *Culex tritaenhyorinchus*. This mosquito is the primary vector of JE, and its abundance is associated with the presence of pigs [47] of rice and other irrigated crops. *Culex tritaenhyorinchus* has been confirmed to transmit many arboviruses found naturally in the field (field incrimination), such as Getah, Rift Valley Fever, Sindbisand Tembusu, especially in Southeast Asia [7]. The discovery of the JE virus in Indonesia was found in *Culex tritaenhyorinchus* in paddy and irrigated areas [48]. *Culex tritaenhyorinchus* also has insect-specific flavivirus, that is, Quang Binh virus (QBV) in Vietnam and the Yunnan Culex Flavivirus (YNCxFv) in China [40].

Culex vishnui and *Culex bitaenhyorinchus* are also a vector of the JE virus, but when compared to *Culex tritaenhyorinchus*, its distribution is limited geographically. *Culex vishnui* was found in all three locations, most abundant in Pasangkayu (n = 834) and North Toraja (n = 319) than Maros (n = 27). This mosquito was also the second-highest after *Culex tritaenhyorinchus*. JE virus was also found in *Culex gellidus* [37] and became the first mosquito to find the Batai virus in Malaysia. This mosquito was found in all three locations and was the most abundant in Pasangkayu (n = 483) and North Toraja (n = 186) than Maros (n = 3). This mosquito needs to be alerted because it has the potential to transmit many types of arbovirus. *Culex sitiens* transmits North Creek Virus (NORCV) (Rhabdovirus), Liao Ning Virus (LNV) (Reovirus), and Ross River Virus (RRV) [42]. *Culex sitiens* was found highest in Pasangkayu (n = 319) compared to Maros (n = 26) and North Toraja (n = 61). JE virus in India was also found in *Culex fuscocephalus* [37], this mosquito, is only found in North Toraja (n = 14). In fact, malaria transmission does not only attack humans but also animals (avian malaria). However, cases have not been reported yet. The potential vectors such as *Culex nigropunctatus, Culex quinqifasciatus, Culex sitiens, Lz. fuscana, Lz. vorax,* and *Uranotaenia* at the sampling sites indicated a risk of transmission of avian malaria. *Culex quinqifasciatus* is found mostly in Maros. This typical mosquito prefers dirty water, stagnates, and around settlements, and this might be the impact of the Pucak river (in Maros) which is dammed due to community development. This mosquito is the main vector of lymphatic filariasis and is found at all sites.

Although both mosquitoes are obtained in small numbers, the spread of the dengue virus is still high. This information is based on data for the last 5 years where dengue is still high in all three locations (Supplementary data 4). Mosquito-borne pathogens, including protozoan parasites, nematodes, and arbovirus, are widely distributed in various mosquito species. Although it is generally known that a particular genus of mosquitoes transmits specific pathogens, it is not limited to that information. With a large number of species in each genus, it is necessary to know each of these species and analyze the potential pathogens that can be transmitted.

Anopheles and *Culex* larvae can be found in places that can accommodate rain around settlements such as ponds, canals, ditches, forest ecosystems, ponds, rivers, and rice fields [49], [50]. *Anopheles* larvae will move freely on the water's edge, while the *Culex* will form a raft and float on the surface. The existence of brackish ponds and swamps in Pasangkayu and irrigation in North Toraja can also be breeding sites for mosquito larvae (Supplementary data 3).

A new virus was found in *Ar. subalbatus* in China, that is, Armigeres subalbatus Totivirus (AsTV) from the totiviridae family [51], this mosquito was found in all three locations in small numbers, *Mansonia uniformis* is known to transmit the zika virus in Southeastern Senegal [52] and West Nile in Mauritania and Senegal [41]. *Mansonia uniformis* was found in North Toraja and Pasangkayu.

In addition to the existence of vectors (including the abundance of species and the extent of breeding places) and social factors (presence of patient hosts), a very influential factor is the vector's interest in visiting humans using the HLC method (measured by HBR value). The higher HBR value indicates a high probability of disease transmission. In Maros and North Toraja, the most biting was *Culex*, while in Pasangkayu, it was *Anopheles*. The most dominant *Culex* species are *Culex quinqifasciatus* and *Culex tritaenhyorinchus*. The abundance of *Culex tritaenhyorinchus* at all three locations seems to be correlated with this high HBR value compared to other species. Meanwhile, *Culex quinqifasciatus* was only obtained from the HLC method and was not found in other traps, and this shows the habit of this mosquito which is anthropophilic and only exists around humans. Its ability to transmit multiple pathogens and its adaptive behavior cause *Culex quinqifasciatus* is known as a smart vector [53].

In Pasangkayu, although the number of *Anopheles* is the least compared to other areas, it has the highest HBR value. It is a big warning for vector control, especially since the very dominant species is *Anopheles barbirostris*, which is the main vector of malaria.

The most influencing factor of potential pathogen transmission or linkage between pathogens and host (humans) is the number of mosquitoes that bite humans. In general, more mosquitoes bite humans in Maros (n = 571) than in Pasangkayu (n = 68) and North Toraja (n = 32). This finding is strongly influenced by the researcher's strategy in positioning the different livestock in each location. In Maros, HLC was conducted near settlements where livestock (cattle) was randomly tied and near settlements (Figure 3a). HLC in Pasangkayu is carried out in settlements where livestock (cattle) are tied up far from the settlement (Figure 3b). Livestock (pigs) in North Toraja are placed behind people's houses and arranged in parallel between settlements and breeding habitats (Figure 3c).

These results indicate a significant effect of livestock position. Livestock can be both an attractant and a barrier for mosquitoes that bite humans. The mosquitoes found in Maros are very abundant. Livestock placement here acts as an attractant because mosquitoes are attracted to livestock and are passed on to settlements. Mosquitoes in Pasangkayu tend to be attracted to livestock scattered far from settlements so that only a few are attracted to humans. Whereas in North Toraja, mosquitoes originating from the breeding grounds will be attracted and stop at livestock and do not enter settlements so that the catch is meager.

This result is the same with research by Hewitt in Pakistan and shows that the presence of cows and goats near humans can increase the likelihood of being bitten by Anophelines [34]. In the Philippines, Russell shows evidence that mosquitoes are more attracted to buffaloes [33], and in Cambodia shows more attracted to cow-baited tents [54]. Thus, livestock will be a barrier if placed precisely in groups between breeding sites and settlements. Factors that need to be considered in using zoo barriers are zoophilic vectors, and livestock should be located away from settlements and arranged in systematic lines.

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Supplementary data

Supplementary data 1: List of mosquito species collected in each site

Species	Maros			North Toraja			Pasangkayu		
	Mosq. collected	HLC	HBR	Mosq. collected	HLC	HBR	Mosq. collected	HLC	HBR
Ae. aegypti	1[a]	0	0.00	0	0	0.00	0	0	0.00
Ae. albopictus	13	2	153.85	4	2	500.00	5	3	600.00
Ae. butleri	0	0	0.00	0	0	0.00	9[c]	0	0.00
Ae. dux	0	0	0.00	0	0	0.00	9[c]	0	0.00
Ae. flavipennis	0	0	0.00	0	0	0.00	10[c]	0	0.00
Ae. linneatopennis	0	0	0.00	2	0	0.00	35	0	0.00
Ae. vexan	361	0	0.00	62	9	145.16	271	1	3.69
Ae. vigilax	0	0	0.00	1	0	0.00	372	0	0.00
Aedes sp.	4	0	0.00	80	0	0.00	349	0	0.00
An. argyropus	0	0	0.00	4[b]	0	0.00	0	0	0.00
An. barbirostris	362	6	16.57	642	0	0.00	46	43	934.78
An. barbumrosus	7	0	0.00	18	0	0.00	25	0	0.00
An. flavirostris	13	0	0.00	0	0	0.00	1	0	0.00
An. kochi	5	0	0.00	251	0	0.00	3	0	0.00
An. maculatus	120	0	0.00	0	0	0.00	35	0	0.00
An. minimus	254[a]	0	0.00	0	0	0.00	5	0	0.00
An. nigerrimus	96	0	0.00	92	0	0.00	0	0	0.00
An. peditaeniatus	0	0	0.00	2	0	0.00	2	0	0.00
An. subpictus	4	0	0.00	0	0	0.00	1	0	0.00
An. sulawesi	0	0	0.00	0	0	0.00	1[c]	0	0.00
An. sundaicus	0	0	0.00	0	0	0.00	147	0	0.00
An. tesselatus	17	0	0.00	6	0	0.00	3	0	0.00
An. vagus	1076	13	12.08	384	1	2.60	139	0	0.00
Cx. bitaeniorhyncus	5	0	0.00	4	0	0.00	4	0	0.00
Cx. fuscocepalus	0	0	0.00	14[b]	0	0.00	0	0	0.00
Cx. gelidus	3	0	0.00	186	0	0.00	483	0	0.00
Cx. hutchinshoni	0	0	0.00	25	0	0.00	3	0	0.00
Cx. infula	0	0	0.00	44[b]	0	0.00	0	0	0.00
Cx. longicornis	0	0	0.00	22[b]	0	0.00	0	0	0.00
Cx. malayi	5	0	0.00	1	1	1000	0	0	0.00
Cx. mimulus	2	0	0.00	0	0	0.00	0	0	0.00
Cx. nigropunctatus	1	1	1000	78	0	0.00	9	0	0.00
Cx.quinqifasciatus	417	417	1000	3	3	1000	5	5	1000
Cx. Sitiens	26	1	38.46	61	8	131.15	319	0	0.00
Cx. tritaeniorhyncus	3195	24	7.51	3782	5	1.32	1954	7	3.58
Cx. vishnui	27	3	111.11	319	0	0.00	834	6	7.19
Cx. whitmorei	1	0	0.00	197	0	0.00	0	0	0.00
Ar. malayi	0	0	0.00	2	2	1000	6	0	0.00
Ar. subalbatus	18	1	55.56	3	1	333.33	2	0	0.00
Cq. crassiper	0	0	0.00	0	0	0.00	1	0	0.00
Lz. fuscana	3	0	0.00	66	0	0.00	0	0	0.00
Lz. vorax	1[a]	0	0.00	0	0	0.00	0	0	0.00
Mn. uniformis	0	0	0.00	15	0	0.00	12	3	250.00
Mi. aurea	0	0	0.00	0	0	0.00	2[c]	0	0.00
Uranotaenia sp.	0	0	0.00	0	0	0.00	1[c]	0	0.00

HLC: Human landing catch; HBR: Human bite rate. Several species collected only from specific districts: [a] Maros, [b] North Toraja, [c] Pasangkayu.

Supplementary data 2: Type of habitats in study sites

Ecology type	Maros	North Toraja	Pasangkayu
	Lowland-forest	Highland-forest	Coastal-forest
Human settlement	X	X	X
Rice field	X	X	0
Irrigation	0	X	0
Forest	X	X	X
Rivers	X	X	X
Brakish pond	0	0	X
Swamp	0	0	X

Supplementary data 3: Number of cases reported for mosquito-borne diseases in study sites, 2016–2020 years (source: South and West Sulawesi Provincial Health Office)

Year	Dengue Fever			Malaria			Filariasis		
	MR	NT	PK	MR	NT	PK	MR	NT	PK
2016	620	96	201	134	158	26	0	1	1
2017	253	29	67	141	119	17	0	1	1
2018	188	57	147	77	172	8	0	1	1
2019	410	14	172	271	NA	5	0	2	0
2020	302	7	148	78	NA	9	0	2	0

MR= Maros, NT = North Toraja, PK = Pasangkayu

Supplementary data 4: List of collected species which were reported harbor pathogens and their habitats

Spesies	Pathogen harbored and references	Habitat
Ae. aegypti	DENV, CHIKV, ZIKV, YFV, W. bancrofti	Highly urbanized, cemented, artificial container, tires
Ae. albopictus	DENVV, CHIKV, ZIKV, YFV, WNV, *W. bancrofti*	Rural, sub-urban, vegetated urban, cemented, artificial container, tires, swamp, bamboo stump, tree hole, animal farm, forest, and lake
Ae. butleri	JEV	Coastal brackish water pools and swamps
Ae. linneatopennis	MVEV, JEV, RVV	Naturally or artificially flooded grassland depressions
Ae. vexan	WSLV, POTV, CHAOV, WNV	Swamp, coastal citrus irrigation, rainfall, or flood irrigation
Ae. vigilax	MVEV, ZIKV, LNV, RRV, SASHV, EHV	Saltmarsh, mangrove basin forest, artificial drainage, tide-affected reticulate area, and mangroves
An. barbirostris	Human *Plasmodium*, *B. malayi, B. timori*	Ditches, ponds, rice fields, tire trail, lagoons, marshes, pools, slow running streams, river, banks, springs, drainage, wells
An. barbumbrosus	Human *Plasmodium*	Swamp, pool, rice fields, river banks, clear streams emerging from jungle areas, open grassy ravines, granite and clay quarry pits, agro wells
An. flavirostris	Human *Plasmodium, W. bancrofti*	River, rice field, irrigation ditches, coastal plains
An. kochi	Human *Plasmodium*	Marsehs, pools, small stream, rice fields, fish ponds, buffalo wallows, wells, ditches, hoof prints
An. maculatus	Human *Plasmodium*, TIBOV, *W. bancrofti*	Stream-side rock pools, margins of small slow-moving streams, drying river beds, ground seepages, small pools, springs, rice fields, ponds, ditches
An. minimus	Human *Plasmodium*, AMIV, *W. bancrofti*	Slow-flowing streams with grassy banks
An. nigerrimus	Human *Plasmodium*	Lake margins, marshes, pools, small streams, rice fields, irrigation channels, large borrow pits, granite and clay quarry pits, agro wells
An. peditaeniatus	JEV	Ditches, ponds, rice field, tire trail, granite and clay quarry pits, agro wells
An. subpictus	Human *Plasmodium, W. bancrofti*	Tidal llagoons, coastal blocked freshwater river and streams, marshes, pools, rocky streams, mangrove, forests, springs, rice fields, fish ponds, furros in gardens, water tanks, buffalo wallows, brackish ponds, irrigation ditches, granite and clay quarry pits
An. sundaicus	Human *Plasmodium*, SFV	Lagoons, marshes, pools, seasonally blocked coastal streams, fish ponds
An. tesselatus	Human *Plasmodium*, KPKV	Ground pools, rice fields, fish ponds
An. vagus	Human *Plasmodium, W. bancrofti*	Ditches, ponds, rice field, tire trail, lagoon, water gutter, stagnants margins of streams, river edges, small and swallow pools near beaches, springs, irrigation, wheel ruts, hoof prints, artificial containers, granite and clay quarry pits, agro wells
Ar. subalbatus	AsTV, *B. pahangi, W. bancrofti, B. malayi*	Stagnant water, tree holes, fecal tank, artificial container, Papaya tree hole, bamboo stump, leaf axils of *Colocasia*, animal farm, forest and lake
Cx. bitaeniorhyncus	JEV, *W. bancrofti*	Rice field, stream pool, irrigation ditch, pond
Cx. fuscocepalus	JEV	Ground pool, hoof-mark, rice field, clay quarry pits
Cx. gelidus	JEV, BATV, GETV, RRV, BFV, KUNV, MVEV, SINBV, TEMV, WNV	Rural areas, paddy fields, cultivated areas, and pig farms, ditches, ponds, rice fields, agro wells, granite quarry pits
Cx. infula	JEV	Granite and clay quarry pits, agro wells
Cx. nigropunctatus	Avian *Plasmodium*	Hoof-mark, ground pool, stream pool
Cx. quinqifasciatus	Avian *Plasmodium*, *W. bancrofti, B. malayi*, WNV, JEV	Urban, suburban, rural, and remote areas, canal, cemented, ditches, drains, ponds, rice fields, water logging cloudy and dirty water near settlements, artificial container, ground pool(55); animal farm, forest and lake
Cx. sitiens	Avian *Plasmodium*, NORCV, LNV, RRV	Brackish fish pond, ground pool, artificial, container
Cx. tritaeniorhyncus	JEV, WNV, GETV, RVFV, SINBV, TEMV, QBV, YNCxFV	Canal, cemented, ditches, drains, ponds, rice fields, water logging, animal farm, forest and lake
Cx. vishnui	JEV	Rural, suburban, rice field, ground pool, irrigation ditch
Lz. fuscana	Avian *Plasmodium*	Stagnant water, artificial container, animal farm, forest and lake
Lz. vorax	Avian *Plasmodium*	Water vase, animal farm, forest and lake
Mn. uniformis	ZIKV, WNV, W. *bancrofti, B. malayi, B. patei*	Ponds, rice fields, swamps, *Eichornia ponds*
Uranotaenia sp.	Avian *Plasmodium*, WNV, NOUV	Bamboo, animal farm, forest and lake

AsTV=*Armigerese subalbatus* totivirus, AMIV=*Anopheles minimus* virus, BATV=Batai virus, BFV=Barmah Forest Virus, CHAOV=Chaoyang virus, CHIKV=Chikungunya virus, DENV=Dengue virus, EHV=Edge Hill virus, GETV=Getah virus JEV=Japanese Enchepalitis virus, KPKV=Kampung Karu virus, KUNV=Kunjin VirusLNV=Liao Ning virus, MVEV=Murray Valley Encheplitis virus, NORCV=North Creek virus, NOUV=Nounane virus, POTV= Potosi virus, QBV=Quang Bingh virus, RRV=Ross River virus, RVFV= Rift Valley Fever virus, SASHV=Salt Ash virus, SFV=Semiliki Forest virus, SINBV=Sindbis virus, TEMV=Tembusu virus, TIBOV=Tibet Orbivirus, WNV = West Nile Virus, WSLV= Wesselsbron virus, ZIKV= Zika virus, YFV=Yellow Fever virus, YNCxFV=Yunnan Culex Flavivirus, YUOV= Yunnan Orbivirus