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 compared 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 Anopheles 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 malayi, 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...
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.

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 where 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),
and Pasangkayu (5103) consisted of 45 species from nine genera: Aedes, Anopheles, Armigeres, Culex, Coquilletidæ, Lutzia, Mansonia, Mimomyia, 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).

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 Anophele collected: Anophele argyropus, Anophele barbirostris, Anophele barbumbrosus, Anophele flavirostris, Anophele kochi, Anophele maculatus, Anophele minimus, Anophele nigerrimus, Anophele peditaeniatus, Anophele subpictus, Anophele sulawesi, Anophele sundaicus, Anophele tesselatus, and Anophele vagus. Culex found in three locations consisted of 14 species, that is, Culex bitaenhyorinchus, Culex fuscocephalus, Culex gellidus, Culex hutchinsoni, Culex infu, Culex longicornis, Culex malayi, Culex miminus, Culex nigropunctatus, Culex sitiens, Culex quinquifasciatus, Culex tritaenhyorinchus, Culex vishnui, and Culex whitmorei. Mosquitoes from other genera (Armigeres, Coquilletidæ, Lutzia, Mimomyia, 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).
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)](https://oamjms.eu/index.php/mjms/index)
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 distinctly 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, Coquito lidtdia 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 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 aegypti 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 (XVF) 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 Potos 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 anthropophilic 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 tesselatus in Sarawak, Malaysia [40]. Anopheles tesselatus 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 other 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 tritaeniyorinchus 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 tritaeniyorinchus. 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 tritaeniyorinchus 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 tritaeniyorinchus in paddy and irrigated areas [48]. Culex tritaeniyorinchus also has insect-specific flavivirus, that is, Quang Binh virus (QBV) in Vietnam and the Yunnan Culex Flavivirus (YNCFv) in China [40].

Culex vishnui and Culex bitaeniyorinchus are also a vector of the JE virus, but when compared to Culex tritaeniyorinchus, 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 tritaeniorhynchus. 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 quinquefasciatus, Culex sitiens, Lz. fuscana, Lz. vorax, and Uranotaenia at the sampling sites indicated a risk of transmission of avian malaria. Culex quinquefasciatus 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 quinquefasciatus and Culex tritaeniorhynchus. The abundance of Culex tritaeniorhynchus at all three locations seems to be correlated with this high HBR value compared to other species. Meanwhile, Culex quinquefasciatus 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 quinquefasciatus 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 Mosq. collected | HLC | HBR | North Toraja Mosq. collected | HLC | HBR | Pasangkayu Mosq. collected | HLC | HBR |
|----------------------|-----------------------|-----|-----|-------------------------------|-----|-----|---------------------------|-----|-----|
| Ae. aeegypti         | 1                     | 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*                        | 0   | 0.00|
| Ae. dux              | 0                     | 0   | 0.00| 0                             | 0   | 0.00| 9*                        | 0   | 0.00|
| Ae. flavipes         | 0                     | 0   | 0.00| 0                             | 0   | 0.00| 10*                       | 0   | 0.00|
| Ae. linneatennis     | 0                     | 0   | 0.00| 0                             | 2   | 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. argyipus         | 0                     | 0   | 0.00| 4*                            | 0   | 0.00| 0                         | 0   | 0.00|
| An. barbirostris     | 362                   | 6   | 16.57| 642                         | 0   | 0.00| 46                        | 43  | 934.78|
| An. barbupressus     | 7                     | 0   | 0.00| 18                            | 0   | 0.00| 25                        | 0   | 0.00|
| An. bivestris        | 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                   | 0   | 0.00| 0                             | 0   | 0.00| 5                         | 0   | 0.00|
| An. nigrimbus        | 96                    | 0   | 0.00| 92                            | 0   | 0.00| 0                         | 0   | 0.00|
| An. pelicinaenius     | 0                     | 0   | 0.00| 2                             | 0   | 0.00| 2                         | 0   | 0.00|
| An. sulpicius        | 4                     | 0   | 0.00| 0                             | 0   | 0.00| 1                         | 0   | 0.00|
| An. sulisaei         | 0                     | 0   | 0.00| 0                             | 0   | 0.00| 1*                        | 0   | 0.00|
| An. sundaeiacus      | 0                     | 0   | 0.00| 0                             | 0   | 0.00| 147                       | 0   | 0.00|
| An. tessellatus      | 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. bitenseifericus  | 5                     | 0   | 0.00| 4                             | 0   | 0.00| 4                         | 0   | 0.00|
| Cx. fuscopheraxus    | 0                     | 0   | 0.00| 14                            | 0   | 0.00| 0                         | 0   | 0.00|
| Cx. gelidus          | 3                     | 0   | 0.00| 186                           | 0   | 0.00| 483                       | 0   | 0.00|
| Cx. huchinsoni       | 0                     | 0   | 0.00| 25                            | 0   | 0.00| 3                         | 0   | 0.00|
| Cx. infula           | 0                     | 0   | 0.00| 44                            | 0   | 0.00| 0                         | 0   | 0.00|
| Cx. longicorius      | 0                     | 0   | 0.00| 22                            | 0   | 0.00| 0                         | 0   | 0.00|
| Cx. malayi           | 5                     | 0   | 0.00| 1                             | 1   | 1000  | 140                        | 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. quinquefasciatus | 417                   | 417 | 1000  | 3                        | 3   | 1000  | 518                        | 5   | 1000|
| Cx. sbiens           | 26                    | 1   | 38.46| 61                          | 8   | 131.15| 319                       | 0   | 0.00|
| Cx. tritesfericus    | 3195                  | 24  | 7.51  | 3782                         | 5   | 1.32 | 1956                      | 7   | 3.58|
| Cx. vishni           | 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|
| M. albipictus        | 0                     | 2   | 2     | 2                            | 1000 | 6    | 0                         | 0   | 0.00|
| M. subalbatus        | 18                    | 1   | 55.56| 3                             | 1    | 333.33| 2                         | 0   | 0.00|
| Cq. crassipier       | 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*                    | 0   | 0.00| 0                             | 0   | 0.00| 0                         | 0   | 0.00|
| Mf. uniformis        | 0                     | 0   | 0.00| 15                            | 0   | 0.00| 12                        | 3   | 250.00|
| Mi. aurea            | 0                     | 0   | 0.00| 0                             | 0   | 0.00| 2*                        | 0   | 0.00|
| Urobonasia sp.       | 0                     | 0   | 0.00| 0                             | 0   | 0.00| 1*                        | 0   | 0.00|

HLC: Human landing catch; HBR: Human bite rate. Several species collected only from specific districts: * Maros, ** North Toraja, *** 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 | 622 | 96 | 201 | 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 | 5 | 0 | 2 | 0 |
| 2020 | 302 | 7 | 148 | 78 | NA | 9 | 0 | 2 | 0 |

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

| Species | Pathogen harbored and references | Habitat |
|---------|----------------------------------|---------|
| Ae. aegypti | DENV, CHIKV, ZIKV, YFV, W. bancrofti | Highly urbanized, cemented, artificial container, tires |
| Ae. albopictus | DENNV, CHIKV, ZIKV, YFV, WN | 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. innenepennis | MVEV, JEV, RVV | Naturally or artificially flooded grassland depressions |
| Ae. vexan | WSLV, POTV, CHAOV, WN | Swamp, coastal citrus irrigation, rainfall, or flood irrigation |
| Ae. vijgale | MVEV, ZIKV, NV, RR, BASHV, EHV | Saltmarsh, mangrove forest basin, artificial drainage, tide-affected reticulate area, and mangroves |
| An. barbirostris | Human Plasmidum, B. malayi, B. timori | Ditches, ponds, rice fields, tree trunks, lakes, mosses, pools, slow running streams, river, banks, springs, drainage, wells |
| An. bartbromus | Human Plasmidum | Swamp, pool, rice fields, river banks, clear streams emerging from jungle areas, open grassy ravines, granite and clay quarry pits, agro wells |
| An. flavinosa | Human Plasmidum, W. bancrofti | River, rice field, irrigation ditches, coastal plains |
| An. kochi | Human Plasmidum | Marshy pools, small stream, rice fields, fish ponds, buffalo wallows, pools, ditches, hoof prints |
| An. marculatus | Human Plasmidum, 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 Plasmidum, AMV, W. bancrofti | Slow-flowing streams with grassy banks |
| An. nigerrimus | Human Plasmidum | Lake margins, marshes, pools, small streams, rice fields, irrigation channels, large borrow pits, granite and clay quarry pits, agro wells |
| An. petrieniatus | JEV | Tidal lagoons, coastal blocked freshwater river and streams, marshes, pools, rocky streams, mango groves, forests, springs, rice fields, fish ponds, furnos in gardens, water tanks, buffalo wallows, brackish pools, irrigation ditches, granite and clay quarry pits |
| An. sudenticus | Human Plasmidum, SFV | Lagoons, marshes, pools, seasonally blocked coastal streams, fish ponds |
| An. tesselatus | Human Plasmidum, KPV | Ground pools, rice fields, fish ponds |
| An. vagus | Human Plasmidum, W. bancrofti | Ditches, ponds, rice field, tree trunks, lagoon, water gutter, stagnants margins of streams, river edges, small and swallow pools near beaches, springs, irrigation, wheel nuts, hoof prints, artificial container, granite and clay quarry pits, agro wells |
| Ar. subalbasus | AsTV, B. pahangi, W. bancrofti, B. malayi | Stagnant water, tree holes, feclal tank, artificial container, Papaya tree hole, bamboo stump, leaf axis of Colocasia, animal farm, forest and lake |
| Cx. bitumorhynchus | JEV, W. bancrofti | Rice field, stream pool, irrigation ditch, pond |
| Cx. fuscocephalus | JEV | Ground pool, hoof-mark, rice field, clay quarry pits |
| Cx. gelidus | JEV, BATV, GETV, RRV, BFV, KUNV, MVEV, SIMNV, TEMV, WN | Rural areas, paddy fields, cultivated areas, and pig farms, ditches, ponds, rice fields, agro wells, granite quarry pits |
| Cx. infusa | JEV | Granite and clay quarry pits, agro wells |
| Cx. nigropunctatus | Avian Plasmidum | Hoof-mark, ground pool, stream pool |
| Cx. quinques. | Avian Plasmidum, W. bancrofti, B. malayi, WN, JEV | Urban, suburban, rural, and remote areas, canals, 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 Plasmidum, NORCV, LNV, RR | Brackish fish pond, ground pool, artificial container |
| Cx. titibanghyreni | JEV, WN, GETV, RR, SIMNV, TEMV, QBV, YNCF | 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 Plasmidum | Stagnant water, artificial container, animal farm, forest and lake |
| Lz. vorax | Avian Plasmidum, WN, NOUV | Water vane, animal farm, forest and lake |
| Mn. uniformis | ZIKV, WN, W. bancrofti, B. malayi, B. patei | Ponds, rice fields, swamps, Echornia ponds |
| Uranotoba sp. | Avian Plasmidum, WN, NOUV | Bamboo, animal farm, forest and lake |

*AsTV=A. subalbasus totivirus, AMIV=Anopheles minimus virus, BATV=Battasi virus, BFV=Bermat Forest Virus, CHAOV=Chao Yang virus, CHIKV=Chikungunya virus, DENV=Dengue virus, EHV=Edge Hill virus, GETV=Gaeth virus, JEV=Japanese Encephalitis virus, KPV=Kampung Karu virus, KUNV=Kunjin Virus, LNV=London virus, MVEV=Murray Valley Encephalitis virus, NORCV=North Creek virus, NOUV=Novum virus, POTV=Potosi virus, QBF=Quan Binh virus, RR=Rose 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=Weeselebron virus, ZIKV=Zika virus, YFV=Yellow Fever virus, YNCxFV=Yunnan Culex Flavivirus, YUOV=Yunnan Orbivirus.*

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