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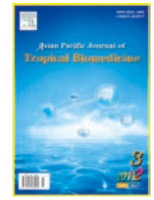

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Unusual developing sites of dengue vectors and potential epidemiological implications

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ABSTRACT

Objective: To identify the unusual breeding sites of two dengue vectors, *i.e. Aedes albopictus* (*Ae. albopictus*) and *Aedes aegypti* (*Ae. aegypti*). **Methods:** During the second half of 2010, we performed an occasional survey in rural (Teluk Tempoyak) and urban (Gelugor) areas of Penang Island, Malaysia, to identify cryptic breeding sites. **Results:** In the rural area, we found heterogeneous immature stages of *Ae. albopictus* in the water bowl of an encaged bird. We also observed *Ae. aegypti* eggs deposited in the flush tank of a toilet in the urban area. **Conclusions:** It can be concluded that both breeding patterns can increase contact with hosts (humans and birds) and presumably population densities of *Ae. albopictus* and *Ae. aegypti*, thereby potentially boosting the risks for spread and transmission of arboviral diseases.

1. Introduction

Originating in Africa[1], *Aedes aegypti* (*Ae. aegypti*) can now be found in many urbanized areas around the world[2,3], due to its ability to breed in habitats close to humans[4]. This characteristic is shared with another particularly invasive mosquito species, *Aedes albopictus* (*Ae. albopictus*) [5,6]. Previously believed to be restricted to South-East Asia forests[7], *Ae. albopictus* has become well established in the western hemisphere[8]. These two *Aedes* mosquito species act as vectors of dengue[9], a human disease caused by one of four closely related but antigenically distinct virus serotypes belonging to the genus *Flavivirus*[10]. About 50%-60% of the projected global population in 2085 is expected be at risk for dengue transmission[11]. To date, an estimated 2.5 billion people are at risk of dengue globally, more than 70% of whom reside in countries in the Asia Pacific region[12].

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In Malaysia, a total of 33 684 people were infected with dengue viruses in 2009[12]. As of October 2010, there have been 40 152 cases and 118 deaths in Malaysia[12]. In this country, *Ae. aegypti* was first found during the 20th century in coastal towns[13]. By 1920, it had already moved inland[14] and has been considered to be main vector of dengue since the early 1950s[7]. Suspected dengue transmission by *Ae. albopictus* was first reported in 1958 by Smith, who also reported that this mosquito was breeding in forest canopies. *Ae. albopictus* is now known to breed inside homes on Penang Island, where *Ae. aegypti* is also present[15].

Measures to control the spread of dengue and related diseases are dependent on how well vector management programs can target the areas where the vectors breed and develop. The main vector, *Ae. aegypti*, is highly anthropophilic[16] and prefers to feed during the day and to rest inside houses. Female *Ae. aegypti* shows a preference for laying their eggs in domestic containers[17], but may also use rainwater-accumulating containers present in peridomestic environments[18,19]. This ability to utilize many containers near human dwelling areas combined with its indoor resting behavior suggests the existence of cryptic microhabitats. However, the variability in domesticity, an important factor in maintaining constant and close contact between a disease vector and its host, has rarely been investigated in *Ae. aegypti*. Previously believed to lack the marked domesticity of *Ae. aegypti*, a second dengue vector, *Ae. albopictus*, has recently been reported adapted to indoor environments on Penang Island[15]. This species is known as an opportunistic and aggressive biter with a wide host range, including humans and a variety of vertebrates[20]. As in many countries of Southeast Asia, rural residents of Malaysia rear birds as pets in their backyards[21]. Certain birds are hosts of many pathogens, such as Chikungunya virus that has been detected in Malaysian patients[22] and which can infect *Ae. albopictus*[23]. Despite these risk factors, which may play a key role in the spread and transmission of arboviral diseases, the effects of keeping birds as pets on the population dynamics of *Aedes* vectors are largely unknown.

Since it was discovered that *Ae. albopictus* has adapted to indoor environments in Penang State in 2009, a change likely to improve its capacity to spread disease, *Aedes* surveys have been conducted during the second half of 2010 in some parts of the island part of Penang State. Located in northern Malaysia, Penang Island has an area of 293 km^2 and lies between north latitudes 5 ° 8′, 5 ° 35′ and east longitudes 100 ° 8′, 100 ° 32′[24]. The island has a typical tropical equatorial climate with an average annual rainfall varying from 267 to 624 cm[24]. Penang Island has two typographically different landscapes-more than half of the west side of the island is forest and hilly, running north and south down the center of the island; a minor plains area with lowlands in the eastern side of the Island is occupied by human populations.

2. Materials and methods

2.1. Surveys

A survey of *Aedes* was conducted in October 2009 in Teluk Tempoyak, located near Penang International Airport. Peridomestic and indoor artificial containers were targeted for the collection of immature stages of *Aedes* mosquitoes. Immature stages of *Aedes* were sampled where found. The water with debris and immature mosquito stages was poured into plastic bags and brought to the laboratory for further sorting and identification. In July 2010, one of the co-authors was bitten by an *Aedes* mosquito in a toilet in the township of Gelugor, the point connecting the Island to the mainland through a bridge 13.5 km in length. Morphological identification using Tanaka's key[25] indicated that the female collected in the toilet was *Ae. aegypti*. This prompted a thorough entomological survey to examine the presence of *Aedes* mosquitoes in the houses surrounding the one where the mosquito was found breeding inside toilets. In houses where householders accepted our inspection request, rooms, toilets, and indoor containers were checked for the presence of any stage of *Aedes* vectors. A sample of eggs was collected and brought to the laboratory.

2.2. Data collection and analysis

In the first survey, the contents of plastic bags were poured into plastic containers (18 cm × 10 cm). Larvae were allowed to develop under the following conditions: temperature (29±3) ℃, relative humidity (75±10)%, and photoperiod 13:10 h with 1 h of dusk. Wild-collected pupae and laboratory pupated individuals were transferred into porcelain bowls filled with 200 mL of tap water and kept in mosquito cages measuring (30 cm × 30 cm × 30 cm). In both surveys 1 and 2, adults were identified morphologically to the species level using a dissecting microscope (Olympus CX41; Olympus, Tokyo, Japan) and the key of Tanaka[25]. In the second survey, eggs were hatched according to the method reported previously by Tanaka[26]. Newly enclosed larvae were reared on a diet of dried yeast under the same environmental conditions. Newly emerged adults had access to 10% glucose solution until processing for identification. As the aim of this study was only to identify unexpected breeding sites of *Aedes* vectors, other known sites were not enumerated.

3. Results

3.1. Survey Aedes in the rural area

Approximately 20 indoor and outdoor containers were inspected. Most of these did not contain water; those that did contain water generally did not harbor immature mosquito stages. In one house, we found a container kept inside a bird cage (Figure 1A and 1B) holding 750 mL of water and heterogenous larval populations and pupae of *Ae. albopictus*. A total of 285 immature mosquitoes were found, including all developmental stages. A total of 88.70% (253/285) of these immature mosquitoes were larvae and the remaining reached the pupal stage. Of these larvae, 9.88% (25/253), 26.50% (67/253), 31.22% (79/253) and 32.41% (82/253) were first instars, second instars, third instars and fourth instars, respectively.

3.2. Survey Aedes in the urban area

Aedes eggs were found inside the flush tank of a toilet in one of four houses where consent was obtained. Morphological identification of adults derived from these eggs revealed that they belonged to *Ae. aegypti*. It was therefore clear that this mosquito used the flush tank of the toilet as an oviposition site (Figure 1C and 1D).

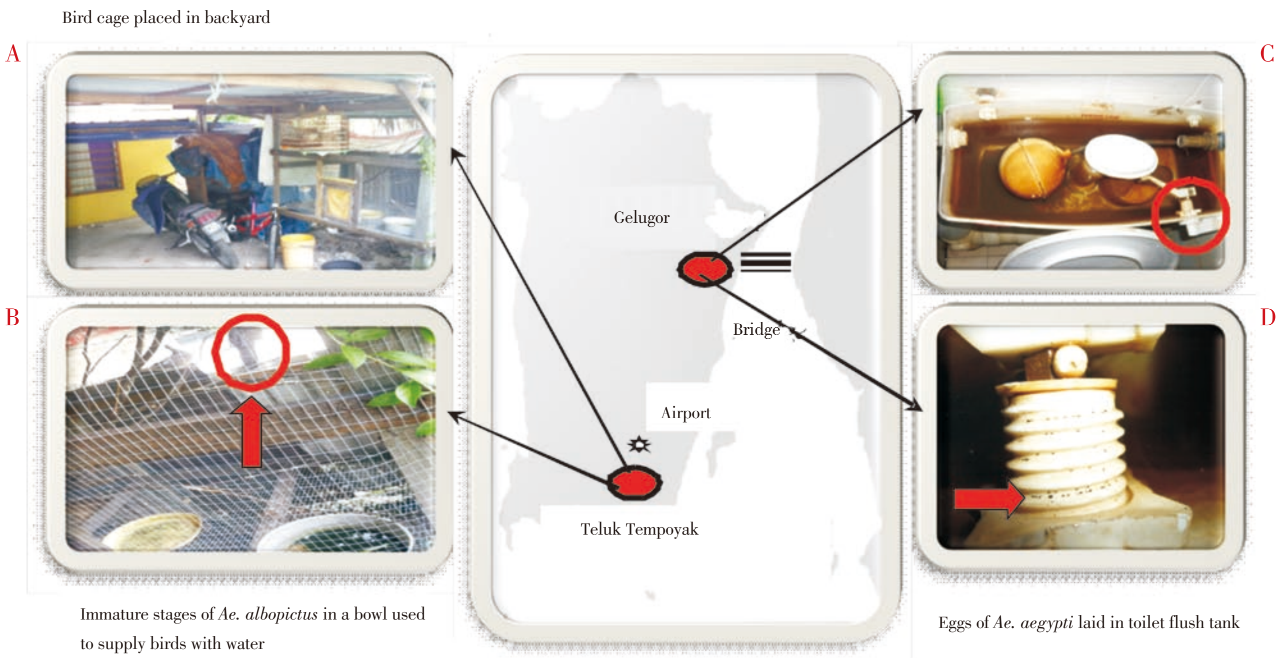

Figure 1 Characteristics of *Aedes* collections from a bird cage container in Teluk Tempoyak and from the flush tank of a toilet in Gelugor. The bold circle and the arrow in Figure 1B designate encaged birds. The arrow in Figure 1D shows the eggs in the interstices of the levee.

4. Discussion

During our entomological survey in October 2010 at Teluk Tempoyak, a rural area of Penang Island, *Ae. albopictus* was noted at various developmental stages in containers kept inside a bird cage. This diversity of developmental stages was likely the result of several egg depositions and has important ecological and epidemiological implications. The presence of immature stages strongly suggests that at least egg deposition had been achieved. It has been reported that female *Ae. albopictus* can discriminate habitats for egg deposition that enhance success for the offspring[27–30]. During the collection of container contents, we observed freshly deposited bird feces on the edges of the container. This suggested that detritus present in the container consisting of bird feces may function as feeding resource for mosquito larvae. In nature, *Ae. albopictus* feeds opportunistically on a variety of vertebrates outdoors[31–33]. It has also been reported that this mosquito occasionally feeds on birds[34,35]. Thus, the presence of developing larvae inside the bird cage can potentially increase the biting activity of emerging adults. These observations may have important implications for disease transmission. Delatte *et al*[20] assessed blood feeding behavior of *Ae. albopictus* on calves, chickens, dogs, goats, and humans. Their results indicated that with equal availability of one of the four vertebrate hosts and human, *Ae. albopictus* showed a significant preference for feeding on humans. They argued that this multiple blood feeding behavior and the preference for humans constitute a high risk for the spread of arboviruses to the human population. *Ae. albopictus* is experimentally receptive to four flaviviruses, ten bunyaviruses, and seven alphaviruses[36,37]. Clearly, within a cage holding birds, emerging females have increased chances of acquiring pathogens, as birds naturally harbor many of these arboviruses[38], such as West Nile and Chikungunya viruses, which are transmissible by *Ae. albopictus*[23,39]. It was recently reported that *Ae. albopictus* is well adapted to the indoor environment within homes in Penang Island, which was suggested to favor long life and increased lifetime reproductive output[15]. Therefore, it is likely that breeding within bird cages will favor long lifespan as blood can serve as an energy source for survival and adaption[40].

Ae. aegypti deposited eggs inside the flush tank of a toilet in Gelugor in 2010. A similar observation was also reported in three other houses in the same area. The presence of *Ae. aegypti* in a bathroom was recently reported in bordering Indonesia[41]. To examine where this mosquito prefers to oviposit, these authors placed ovitraps in several locations within peoples' houses in West Java and found that *Ae. aegypti* showed increased egg deposition activity in bathrooms. Their study, which lasted for one month, also showed that the larvae of this mosquito can develop in toilet environments. In their study, larvae were sampled for laboratory identification about one week post-eclosion. Our survey was prompted by the presence of a biting adult *Ae. aegypti* within a toilet in Gelugor, an urban area on Penang Island, Malaysia. Although we did not determine whether the eggs found inside the flush tank could hatch and develop in the toilet environment, the important point to note was that we collected a biting adult from the toilet. This observation combined with the presence of larvae developing in bathrooms in neighboring Indonesia[41], strongly suggests that *Ae. aegypti* is capable of breeding in the toilet environment. Certain conditions found in toilet environments are conducive to the presence of *Aedes* mosquitoes. In general, mosquitoes breeding in containers show a preference for drought-resistant ones[42]. In a functional toilet, water is always present in the flush tank. Oviposition responses, egg viability[26], and adult population size[43] are increased under high moisture conditions. The presence of water in the toilet flush tank and showers results in increased relative humidity. Adaptation to the toilet environment results in an increase in human-vector contact. *Ae. aegypti* is known to exhibit multiple blood feeding activity[44], presumably in response to host defense levels. This mosquito also exhibits increased blood feeding frequency when a host is available[15]. Such blood feeding characteristics are likely to be expressed within a toilet. Environment-toilets are places where people usually remain only for short periods and uncover parts of their body, thereby increasing blood feeding opportunities for mosquitoes. Our observations have implications for population persistence of dengue vectors in Penang's rural and urban areas and for arboviral disease transmission. The permanent presence of water is positively correlated with the abundance of container-breeding *Aedes* mosquitoes[45]. Thus, *Ae. aegypti* breeding in toilet flush tanks and *Ae. albopictus* developing in caged bird water bowls are expected to have population maintenance as both sites usually contain water. Other studies have shown that mosquitoes, including *Aedes* surveyed here, feed on blood for immediate energy needs, but in some cases, blood is used as an alternative energy source for survival[46]. In addition, *Ae. aegypti* and *Ae. albopictus* can exhibit multiple blood feeding behaviors. If expressed, these features may increase opportunities for the transfer of arboviruses between the mosquitoes and their avian or humans hosts. *Ae. albopictus* breeding and *Ae. aegypti* ovipositing in a toilet flush tank were observed in only one and three houses, respectively, and we do not yet know the extents of these breeding patterns. Additional surveys are therefore required to determine whether these breeding patterns occur to a large extent. In particular, the observation of developing *Ae. albopictus* in the water bowl inside a bird cage where water is generally available warrants further research regarding the effects of this phenomenon on the blood feeding habits and the presence of viruses transmissible by this mosquito in pet birds. Such knowledge could provide insight into the vector potential of this mosquito.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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