Scientific Note

Pre-imaginal development of *Aedes aegypti* in brackish and fresh water urban domestic wells in Sri Lanka

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There is a long and widely held view that *Aedes aegypti* (Linnaeus) and *Ae. albopictus* Skuse (Diptera: Culicidae), the principal mosquito vectors of arboviruses causing yellow fever, dengue, and chikungunya, oviposit and undergo pre-imaginal development only in freshwater collections near human habitations (Barraud 1934, Weaver and Reisen 2010, Walter Reed Biosytematics Unit 2012, World Health Organization 2009). Larval source reduction efforts worldwide, therefore, focus on freshwater habitats of the two vectors. However, *Ae. aegypti* and the closely related arboviral vector *Ae. albopictus* were recently shown to also undergo pre-imaginal development in brackish water of up to 15 ppt salt (water with <0.5 ppt or parts per thousand salt is fresh, 0.5–30 ppt salt brackish, and >30 ppt salt saline) in discarded food and beverage containers, as well as abandoned wells and boats, along the coast of the Batticaloa and Jaffna districts in tropical Sri Lanka (Ramasamy et al. 2011). Dengue is endemic to Sri Lanka with 28,473 cases and 185 deaths in 2011. There were 400 dengue cases with four deaths and 1,693 cases with 11 deaths, respectively, in 2011 in the Jaffna and Batticaloa districts of the country. The Jaffna district also experienced an epidemic of chikungunya in 2006-2007 (Surendran et al. 2007). Dengue is of global health concern because of its increasing incidence and spread, associated mortality and morbidity, and the lack of a specific drug or vaccine (World Health Organization 2009). Furthermore, *Ae. albopictus* has adapted to temperate zones to transmit chikungunya and dengue in Europe (Cavrini et al. 2009, Rezza et al. 2007, La Ruche et al. 2010). Climate change can increase the future global incidence and spread of dengue and other arboviral diseases transmitted by *Ae. aegypti* and *Ae. albopictus* (Hales et al. 2002, Ramasamy and Surendran 2011, 2012, Reiter 2001,Weaver and Reisen 2010).

In many tropical developing countries, wells are an important source of water for drinking, washing, and other household uses. Wells near the Jaffna coast, Sri Lanka, tend to be brackish and therefore nearly all coastal areas of Jaffna city receive piped fresh water drawn from artesian wells located in Thirunelvely in the center of the Jaffna Peninsula (Figure 1a). The piped fresh water is used for drinking and cooking. Brackish water drawn from wells along the Jaffna coast is used for bathing, washing, watering gardens, and household cleaning. Domestic wells are not targeted by the vector control program in Sri Lanka, and therefore constitute potentially unappreciated habitats for pre-imaginal development of *Ae. aegypti* and *Ae. albopictus*. We tested a hypothesis that the two *Aedes* vectors also develop in frequently used brackish and fresh water domestic wells in dengue-prevalent urban coastal areas of Sri Lanka.

Larval surveys were conducted between September, 2011 and January, 2012 in Kurunagar, a municipal division in the southern coast of Jaffna city, Sri Lanka (Figure 1a), which had an estimated dengue incidence of eight cases per 1,000 inhabitants per year in the period October, 2010 to April, 2011 (Ramasamy et al. 2011). Randomly selected households in Kurunagar were inspected for the presence of *Aedes* larvae in frequently used domestic wells. The objectives of the study were explained to the heads of households and their informed consent obtained to inspect wells. Larvae were collected using a 350 ml capacity dipper with an average of five dips per well. Larvae in collected well water were brought to the laboratory, and salinity determined with a refractor-salinometer (Atago, Japan), and larvae identified with standard morphological keys (Rueda 2004). The wells were also visually inspected for the presence of fish during the study. A similar survey was conducted in January, 2012 in Kattankudy, a densely populated coastal town in the Batticaloa district of mainland Sri Lanka ~ 350 km from Jaffna city (Figure 1b).

Larvae were subsequently collected from five brackish water wells (salinity range of 2 to 4 ppt) in Kurunagar in May 2012 and reared in the corresponding well water in the laboratory until they became adults that were then identified morphologically (Rueda 2004). Larvae were fed three times a day with locally available fish meal pellet powder.

Statistical associations between the presence of fish and *Aedes* larvae in wells were determined by Fisher's exact test using pooled data from Kurunagar and Kattankudy. The larval and water sampling data were used to create maps with ArcMap 9.1 software.

Of the 110 wells surveyed in Kurunagar, 28 contained *Ae. aegypti* larvae (Figure 1a). Larval density (mean number/ 350 ml) varied from 2-20. *Aedes albopictus* was not detected in the wells. The salinity in wells that contained *Ae. eagypti* larvae varied from 2 to 9 ppt and in wells that did not have larvae from 2 to 7 ppt. Thirty-seven of the 110 wells were found to contain fish but none of the wells with fish had *Ae. aegypti* larvae. Three fish specimens collected in Kurunagar were identified as *Oreochromis mossambicus* (Tilapia).

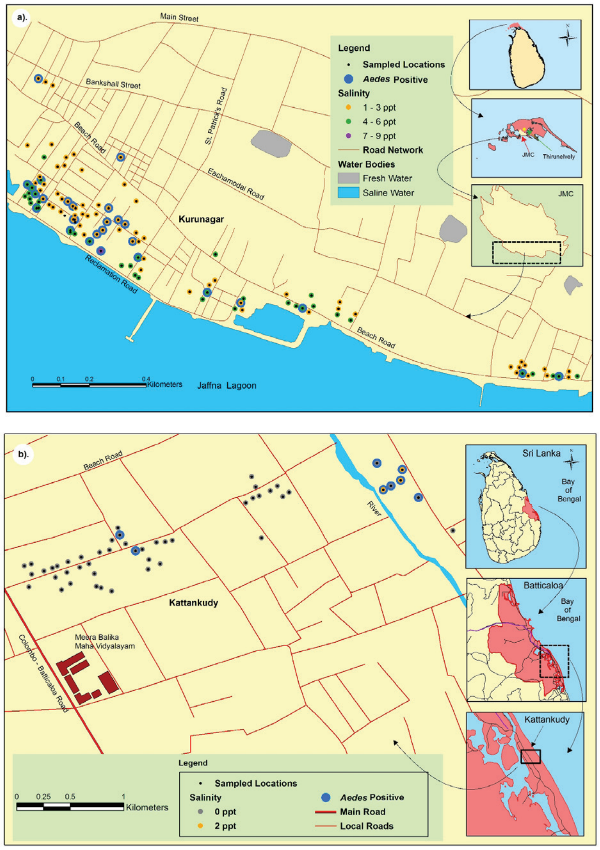

Figure 1. Map showing the study sites. a). Southern part of Jaffna Municipal Council (JMC). b). Eastern coastal part of Kattankudy town.

Of the 50 domestic wells inspected in Kattankudy, 47 wells had freshwater (0 ppt salinity) and three brackish water of 2 ppt salinity (Figure 1b). Larval density varied from 3-9 per 350 ml. Five fresh water and the three brackish water wells in Kattankudy had *Ae. aegypti* larvae (Figure 1b). Seven of the 50 wells inspected at Kattankudy had fish but *Ae. aegypti* larvae were not observed in the wells with fish. *Aedes albopictus* larvae were not observed in the 50 wells inspected in Kattankudy. Overall, in both locations, five of 47 fresh water wells (11%) and 31 of 117 brackish water wells (26%) had *Ae. aegypti* larvae. A one-tailed Fisher's exact test showed that the presence of fish in wells containing either fresh or brackish water at the two locations was significantly associated with the absence of larvae in wells ($p < 0.001$). When 88 *Ae. aegypti* larvae collected from five brackish water wells of 2-4 ppt salinity in Kurunagar were reared separately in the corresponding well water, 82 or 93% emerged as adults.

The control of dengue worldwide relies mainly on active surveillance for dengue cases and vector densities, elimination or reduction of pre-imaginal development habitats of the *Aedes* vectors and the use of insecticides (World Health Organization 2009). Discarded food and beverage containers, tires, and water storage receptacles containing fresh water are recognized as important habitats for pre-imaginal development of *Ae. aegypti* and *Ae. albopictus* in Sri Lanka (Kulatilaka and Jayakuru 1998, Kusumawathie 2005) and other countries (World Health Organization 2009). There have been previous reports of the pre-imaginal development of *Ae. aegypti* in fresh water wells in Australia (Russell et al. 1993), India (Paniker et al. 1982) and Vietnam (Nam et al. 1998). An earlier limited study of domestic wells along the main road from Jaffna city to Thirunelvey did not detect *Aedes* larvae in the wells (Ramasamy et al. 2011). However, that study did not focus on areas with a high incidence of dengue or examine the presence of fish in wells. Health authorities regularly inspect households in Kurunagar for the presence of *Aedes* larvae in freshwater collections in containers. However, domestic wells are not inspected or treated with larvicides in Kurunagar or Kattankudy. The prevalence rates for *Ae. aegypti* larvae in brackish and fresh water wells in the two study sites are compatible with Breteau and House indices that permit high rates of dengue transmission elsewhere (Sanchez et al. 2006). This suggests that pre-imaginal development of *Ae. aegypti* larvae in such habitats may have contributed, in a previously unrecognized manner, to dengue transmission in Kurunagar and Kattankudy. The urban nature of the surveyed areas and the known preference of *Ae. albopictus* for rural and peri-urban environments (Barraud 1934) may explain why *Ae. albopictus* was not detected in the wells in Kurunagar and Kattankudy. The findings imply that the control of dengue and other arboviral diseases transmitted by *Ae. aegypti* in Sri Lanka and other endemic countries requires an extension of larval source reduction and management programs to domestic wells.

The presence of fish in the wells was associated with the absence of *Ae. aegypti* larvae probably due to predation. *Arius* spp (cat fish), *Anabas* spp (climbing perch), and *Oreochromis* spp (tilapia) are commonly found in fresh water bodies including wells in Sri Lanka (Munro 1955, Surendran et al. 2008). Tilapia and climbing perch are often introduced into domestic wells in Jaffna and Batticaloa. Because wells are an essential source of water for many purposes in Jaffna and Batticaloa, the introduction of larvivorous fish such as *Oreochromis mossambicus* (tilapia) that have previously been shown to control *Ae. aegypti* larvae in water tanks in Jaffna with a high predatory index of 240 (Surendran et al. 2008) may be effective in an integrated dengue vector control program.

Our results show for the first time that frequently used brackish water domestic wells, with salinity up to 9 ppt and 2 ppt in the Jaffna and Batticaloa districts, respectively, are suitable habitats for the pre-imaginal development of *Ae. aegypti* in tropical coasts. The salinity tolerance observed is consistent with the detection of larvae in other environmental habitats with salinity up to 15 ppt and 8 ppt and the LC_{50} for salinity in 1^{st} instar larvae of 10 ppt and 4 ppt, respectively, reported previously for *Ae. aegypti* in the Jaffna and Batticaloa districts (Ramasamy et al. 2011). The results also suggest that fresh water domestic wells may be less conducive to *Ae. aegypti* development than brackish water wells. Because the sampled wells in Kurunagar had exclusively brackish water while those in Kattankudy had predominantly fresh water, further studies examining the many variables involved are needed to establish such a possible relationship. Salinity-tolerant *Ae. aegypti* developing in brackish water coastal wells can make an increasing contribution to the transmission of dengue and chikungunya in a future context of rising sea levels, causing an increase in salinity in the Jaffna peninsula and other coastal tropical areas (Ramasamy and Surendran 2011, 2012). Detailed studies are needed to determine the relative importance of *Ae. aegypti* developing in brackish and fresh water domestic wells for dengue transmission in tropical coasts.

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