Salinity-tolerant larvae of mosquito vectors in the tropical coast of Jaffna, Sri Lanka and the effect of salinity on the toxicity of Bacillus thuringiensis to Aedes aegypti larvae

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Abstract

Background: Dengue, chikungunya, malaria, filariasis and Japanese encephalitis are common mosquito-borne diseases endemic to Sri Lanka. Aedes aegypti and Aedes albopictus, the major vectors of dengue, were recently shown to undergo pre-imaginal development in brackish water bodies in the island. A limited survey of selected coastal localities of the Jaffna district in northern Sri Lanka was carried out to identify mosquito species undergoing pre-imaginal development in brackish and saline waters. The effect of salinity on the toxicity of Bacillus thuringiensis israelensis larvicide to Ae. aegypti larvae at salinity levels naturally tolerated by Ae. aegypti was examined.

Methods: Larvae collected at the selected sites along the Jaffna coast were identified and salinity of habitat water determined in the laboratory. The LC50 and LC90 of B. thuringiensis toxin, the active ingredient of a commercial formulation of the larvicide BACTIVEC®, were determined with Ae. aegypti larvae. Bioassays were also carried out at salinities varying from 0 to 18 ppt to determine the toxicity of Bacillus thuringiensis to fresh and brackish water-derived larvae of Ae. aegypti.

Results: Larvae of four Anopheles, two Aedes, one Culex and one Lutzia species were collected from brackish and saline sites with salinity in the range 2 to 68 ppt. The LC50 and LC90 of B. thuringiensis toxin for the second instar larvae of Ae. aegypti in fresh water were 0.006 ppm and 0.013 ppm respectively, with corresponding values for brackish water populations of 0.008 and 0.012 ppm respectively. One hundred percent survival of second instar fresh water and brackish water-derived Ae. aegypti larvae was recorded at salinity up to 10 and 12 ppt and 100% mortality at 16 and 18 ppt, yielding an LC50 for salinity of 13.9 ppt and 15.4 ppt at 24 h post-treatment respectively for the two populations. Statistical analysis showed significantly reduced toxicity of B. thuringiensis to fresh and brackish water-derived Ae. aegypti larvae at high salinities.

Conclusion: A variety of mosquito vectors of human diseases undergo pre-imaginal development in brackish or saline waters in coastal areas of the Jaffna district in northern Sri Lanka. Salinity has a small but significant negative impact on the toxicity of B. thuringiensis toxin to Ae. aegypti larvae at salinity levels where Ae. aegypti larvae are found in the environment. This has implications for the use of B. thuringiensis toxin as a larvicide in brackish waters.

Keywords: Aedes aegypti, Bacillus thuringiensis, Dengue, Jaffna, Mosquito vectors, Salinity, Sri Lanka
Background

*Aedes aegypti* (Linnaeus) and *Ae. albopictus* Skuse are the established vectors of dengue and chikungunya in populated areas worldwide [1-3]. Dengue is of major public health concern in many tropical and semi-tropical countries [1]. In the tropical Jaffna district of northern Sri Lanka, there were 400 cases of dengue with 4 deaths in 2011 [4] and an epidemic of chikungunya in the period 2006 to 2007 [5]. Malaria has been historically endemic in Sri Lanka but its incidence has drastically declined in recent years [6]. Other important mosquito-borne diseases prevalent in Sri Lanka are filariasis [7] and Japanese encephalitis (JE) [8].

In 2011 [4] and an epidemic of chikungunya in the period from August 2011 to May 2012. Kurunagar, Pannai bridge, Delft island and Nainativu were chosen randomly for studying islands. Additionally, two readily accessible mangrove locations viz. at Sarasalai and Pannai bridge were selected from four other mangrove sites in the district. Collections were carried out monthly at each site. Three hundred and fifty ml capacity dippers were used to collect larvae from disused boats, pits, domestic wells, ponds and stagnant water bodies that were considered to be potentially brackish (except during the monsoon season) along the coast. Five dips per collection site on average were performed from 0800 to 1000 h. The collected larvae in water were brought to the Zoology Laboratory of the University of Jaffna at Thirunelvelly, and the salinity of the water determined with a refractometer (Atago, Japan). Larvae were reared to adulthood in the respective collected water as described previously [10]. Initially for rearing purposes, *Aedes*, *Culex* and *Lutiza* larvae and later emergent adults of all mosquito species were identified using standard keys [14-16].

**Methods**

**Mosquito collections**

Mosquito larvae were collected at five coastal locations viz. Sarasalai, Kurunagar, Pannai bridge, Delft island and Nainativu island (Figure 1A-F) in the Jaffna district during the period from August 2011 to May 2012. Kurunagar was selected based on the previous reports [10,11] that pre-imaginal stages of dengue vectors are found in brackish water in the area. Two populated islands off the Jaffna coast viz. Delft and Nainativu were chosen randomly for studying islands. Additionally, two readily accessible mangrove locations viz. at Sarasalai and Pannai bridge were selected from four other mangrove sites in the district. Collections were carried out monthly at each site. Three hundred and fifty ml capacity dippers were used to collect larvae from disused boats, pits, domestic wells, ponds and stagnant water bodies that were considered to be potentially brackish (except during the monsoon season) along the coast. Five dips per collection site on average were performed from 0800 to 1000 h. The collected larvae in water were brought to the Zoology Laboratory of the University of Jaffna at Thirunelvelly, and the salinity of the water determined with a refractometer (Atago, Japan). Larvae were reared to adulthood in the respective collected water as described previously [10]. Initially for rearing purposes, *Aedes*, *Culex* and *Lutiza* larvae and later emergent adults of all mosquito species were identified using standard keys [14-16].

**Evaluation of the toxicity of *B. thuringiensis* at different salinity levels**

Solutions with salinity of 0, 2, 4, 6, 8, 10, 12, 14 and 16 ppt solutions were prepared by adding tap water to sea water as described previously [10]. Second instar larvae of *Ae. aegypti* from a laboratory colony derived from eggs oviposited in fresh water were used for this experiment.
Ten larvae were introduced into each concentration of test solution in 100 ml tap water in 150 ml capacity plastic cups. Tap water alone was used as control. In one set of tests, 0.018 ppm of *B. thuringiensis* toxin (the lowest tested concentration that was found to cause 100% mortality among second instar larvae of fresh and brackish water-derived *Ae. aegypti*) was introduced into all test solutions of varying salinity. Test solutions without BACTIVEC® were used in a second set of tests. Three replicates were run in parallel at each salinity level for tests with and without BACTIVEC®. Mean larval mortality was determined at 24, 48 and 72 h post-treatment. Larvae were fed twice a day with powdered fish meal pellet for the duration of the experiments. A similar experiment at salinities of 0, 2, 4, 6, 8, 10, 12, 16 and 18 ppt was conducted with brackish water-derived *Ae. aegypti* larvae. Mortality in second instar larvae was determined at 24, 48 and 72 h post-treatment with and without BACTIVEC®.
Statistical analysis
The required LC50 and LC90 values with 95% confidence intervals were determined by Probit analysis. The toxicity of *B. thuringiensis* at different salinity levels for each time interval was determined by a two-way ANOVA. The statistical significance of differential survival of fresh water and brackish water-derived *Ae. aegypti* larvae in the presence of BACTI-VEC*®* at the salinity levels of 10, 12, 14 and 16 ppt was determined using the Student’s t-test. All analyses were done using Minitab statistical software (Minitab Inc, PA, USA).

Results
Larvae of four *Anopheles*, two *Aedes*, one *Culex* and one *Lutzia* species were collected from brackish and saline sites with salinity in the range of 2 to 68 ppt. Details of the collected larvae and their habitats are presented in Table 1. The LC50 and LC90 of *B. thuringiensis* for the

Table 1 Brackish water breeding mosquitoes in Jaffna district

| Species          | Transmitted diseases/medical significance          | Location of larval collection | Period of larval collection | Nature of habitat(numbers of sites with larvae) | Average larval number per site in 350ml/dip | Salinity range of brackish water habitats with larvae (ppt) |
|------------------|---------------------------------------------------|-------------------------------|-----------------------------|---------------------------------|------------------------------------------|------------------------------------------------|
| *Aedes aegypti*  | Dengue, Chikungunya                              | Kurunagar                     | 2011 Sep – 2012 May         | Used well (27)                  | 6                                       | 2-9                                            |
|                  |                                                   | Kurunagar                     | 2011 Sep                    | Barrel (1)                      | 35                                      | 10                                             |
| *Aedes albopictus* | Dengue, Chikungunya                            | Sarasalai                     | 2012 Jan                    | Tube near brackish water mangrove marsh (1) | 10                                      | 4                                              |
|                  |                                                   | Sarasalai                     | 2012 Jan                    | Battery box in brackish water mangrove marsh (1) | 8                                       | 4                                              |
| *Anopheles subpictus* | Malaria                                      | Delft                         | 2012 Feb-April              | Pond (2)                        | 14                                      | 2-6                                            |
|                  |                                                   | Nainativu                     | 2012 April                  | Used well (20)                  | 17                                      | 4-39                                           |
|                  |                                                   | Passailoor                    | 2011 Aug                    | Disused boats (2)               | 4                                       | 15-18                                          |
|                  |                                                   | Kurunagar                     | 2011 Sep                    | Used well (3)                   | 7                                       | 2-6                                            |
|                  |                                                   | Sarasalai                     | 2012 Jan                    | Pit in brackish water mangrove marsh (2) | 11                                      | 10-12                                          |
|                  |                                                   | Pannai bridge                 | 2012 May                    | Brackish water mangrove marsh (1) | 3                                       | 10                                             |
| *Anopheles barbirostris* | Malaria                                    | Delft                         | 2012 April                  | Used well (3)                   | 4                                       | 5-6                                            |
|                  |                                                   | Sarasalai                     | 2012 Jan                    | Pit near brackish water mangrove marsh (1) | 2                                       | 4                                              |
|                  |                                                   | Nainativu                     | 2012 April                  | Used well (1)                   | 3                                       | 15                                             |
| *Anopheles varuna* | Malaria                                       | Delft                         | 2012 Jan-April              | Used well (11)                  | 4                                       | 2-4                                            |
| *Anopheles culicifacies* | Malaria                                    | Delft                         | 2012 Jan-April              | Used well (3)                   | 3                                       | 2-4                                            |
|                  |                                                   | Sarasalai                     | 2012 Jan                    | Brackish water mangrove marsh (1) | 2                                       | 4                                              |
| *Culex sitiens*  | Japanese encephalitis, Ross River fever and filariasis | Kurunagar                     | 2011 Sep                    | Disused boats (1)               | 35                                      | 20                                             |
|                  |                                                   | Kurunagar                     | 2011 Sep-2012 Jan           | Used wells (2)                  | 28                                      | 2-6                                            |
|                  |                                                   | Nainativu                     | 2011 Aug - 2012 May         | Used wells (18)                 | 24                                      | 2-39                                           |
|                  |                                                   | Sarasalai                     | 2012 Jan                    | Pit near brackish water mangrove marsh (1) | 6                                       | 12                                             |
|                  |                                                   | Delft                         | 2012 March                  | Pond (5)                        | 9                                       | 4-6                                            |
|                  |                                                   | Pannai bridge                 | 2012 May                    | Brackish water mangrove marsh (1) | 5                                       | 68                                             |
| *Lutzia fuscanus* | Not a vector but feeds on larvae of mosquito vectors | Nainativu                     | 2011 Oct                    | Used well (1)                   | 5                                       | 10                                             |
second instar larvae of fresh water-derived *Ae. aegypti* were 0.006 (95% confidence interval or CI: 0.003 - 0.009) ppm and 0.013 (95% CI: 0.009 – 0.021) ppm respectively. For 10 ppt brackish water-derived *Ae. aegypti*, the LC50 and LC90 values were 0.008 (95% CI: 0.006- 0.011) ppm and 0.012 (95% CI: 0.010 – 0.019) ppm respectively (Figure 2). These results do not demonstrate a significant difference in sensitivity to BACTIVEC® in the two *Ae. aegypti* populations.

The effects of *B. thuringiensis* toxin at varying levels of salinity on the survival of fresh and brackish water-derived *Ae. aegypti* second instar larvae are presented in Figure 3. With increasing salinity in the absence of *B. thuringiensis* toxin, 100% survival of second instar fresh water-derived *Ae. aegypti* larvae was recorded at salinity up to 10 ppt and 100% mortality at 16 ppt with LC50 and LC90 values of 13.9 (95% CI: 13.1-14.8) and 15.4 (95% CI: 14.5 – 17.8) ppt respectively at 24 h post-treatment (Figure 3). Statistical analysis revealed a significant effect of salinity on the lethality of *B. thuringiensis* toxin at each time point (F= 49.03, P=0.00 for 24 h; F= 38.55, P=0.00 for 48 h and 72 h). However, the brackish water-derived larvae showed 100% survival up to 12 ppt salinity and 100% mortality at 18 ppt salinity with LC50 and LC90 values of 15.4 (95% CI: 14.5 – 16.2) and 17.1 (95% CI: 16.2-19.4) ppt respectively at 24 h post-treatment (Figure 3). Although brackish water-derived *Ae. aegypti* larvae tended to have higher LC50 and LC90 values for salinity than fresh water-derived *Ae. aegypti* larvae, the differences are not significant at the P=0.5 level. Statistical analysis however, demonstrated a significant effect of salinity on the toxicity of *B. thuringien-
sis* in 10 ppt salinity at each time point (F=45.38, P=0.00 for 24 h; F=38.68, P=0.00 for 48 h and 72 h) with brackish water-derived larvae as in the case of fresh water-derived larvae. In comparison to freshwater-derived *Ae. aegypti* larvae, the brackish water-derived larvae showed significantly enhanced survival at 14 and 16 but not 10 and 12 ppt salinity in the presence of BACTIVEC® at 24, 48 and 72 h post-treatment (p<0.05 by the Student’s t test, Figure 3).

**Discussion**

The results show that several different mosquito vectors in coastal areas of northern Sri Lanka can undergo pre-imaginal development in collections of brackish and saline water in the environment. Larvae of the known malaria vectors, *Anopheles varuna* and *Anopheles barbirostris* were detected for the first time in brackish water in Sri Lanka. Although *An. culicifacies s.l.*, the major malaria vector in Sri Lanka, is recognized as a fresh water mosquito, it has been recently reported to undergo pre-imaginal development in brackish water of up to 4 ppt in eastern Sri Lanka [17]. The present findings show that this is also the case in the northern Jaffna district in Sri Lanka. *Culex sitiens* is a well-known salinity-tolerant mosquito vector of Japanese encephalitis (JE) virus [18,19] but its role in disease transmission in Sri Lanka has not established. Japanese encephalitis in Sri Lanka is considered to be mainly transmitted by *Culex tritaeniorhynchus* and *Culex gelidus* and occurrence of the disease is associated with rice cultivation and piggeries with a high incidence reported from the inland North-central province of the country [20,21]. Since pigs are also reared in coastal areas of Jaffna city, there is a potential for *Cx. sitiens* to transmit Japanese encephalitis in the Jaffna district.

Although morphologically characterized *An. subpictus s.l.* was detected to undergo pre-imaginal development in brackish and saline water in this study, its taxonomic status in Sri Lanka is doubtful as molecular characterization of ribosomal DNA revealed that most, if not all morphologically characterized *An. subpictus* species B in coastal eastern Sri Lanka are in fact *An. sundaicus s.l.* [22]. Furthermore, results of phylogenetic analysis based on the

![Figure 2](http://www.parasitesandvectors.com/content/5/1/269)  
**Figure 2** Toxicity of *Bacillus thuringiensis* israelensis H-14 toxin against fresh and brackish water-derived second instar *Ae. aegypti* larvae at 24 h post-treatment. The bars show standard errors of the mean.
DNA sequence of the internal transcribed spacer-2 (ITS-2) of the ribosomal RNA gene of two An. subpictus s.l. samples collected from Nainativu island during the study period showed genetic similarity to An. sundaisicus s.l. (sequence data not shown). Anopheles subpictus species B/An. sundaisicus species are well known vectors of dengue and chikungunya. Aedes albopictus has recently been demonstrated in the Jaffna and Batticaloa districts of Sri Lanka [23]. Therefore, considering the well-known salinity tolerance and vector potential of An. sundaisicus s.l. elsewhere in Asia [19], its probable presence in coastal and inland areas of Jaffna peninsula [23] with a higher sporozoite rate than An. culicifacies s.l. [24]. Therefore, considering the well-known salinity tolerance and vector potential of An. sundaisicus s.l. elsewhere in Asia [19], its probable presence in coastal and inland areas of the Jaffna peninsula indicates a potential for malaria transmission. Brackish water development of Ae. aegypti and Ae. albopictus has recently been demonstrated in the Jaffna and Batticaloa districts of Sri Lanka [10]. Ae. aegypti and Ae. albopictus are well known vectors of dengue and chikungunya. Aedes albopictus can also transmit the JE virus [19]. The present findings confirm that the two arboviral vectors are able to undergo pre-imaginal development in brackish water collections in the environment. An interesting observation is that the predatory mosquito Lutzia fuscans which readily feed on other mosquito larvae, especially Aedes larvae (Jude, P.J., Paramsothy, S., Thavaranjith, A.C., Ramasamy, R., Surendran, S.N.; unpublished data), develops in the same brackish water as its prey in Nainativu island. This is the first report of L. fuscans larvae in brackish water in Sri Lanka. Lutzia fuscans is generally regarded to undergo pre-imaginal development in ground-water habitats with a high organic content [25]. The results suggest the L. fuscans may naturally limit the pre-imaginal development of mosquito vectors in brackish water habitats in the Jaffna district.

A previous study that investigated the salinity tolerance of Ae. aegypti and Ae. albopictus in Jaffna district showed that the LC$_{50}$ values for first and third instar fresh water-derived larvae of Ae. aegypti developing into adults were 11.9 and 15.5 ppt salinity respectively [10], consistent with the LC$_{50}$ of 13.9 ppt salinity observed with fresh water-derived second instar larvae at 24 h in the present study. The corresponding values for fresh water-derived Ae. albopictus were reported to be 13.0 and 16.0 ppt in an earlier study [10]. The range of salinities observed in the habitats where Ae. aegypti and Ae. albopictus were collected in the present study are consistent with previous [10] and present laboratory results for salinity tolerance of their larvae.

The present study shows that the salinity levels that Ae. aegypti and Ae. albopictus larvae are able to tolerate in the environment has a small but significant impact on reducing the toxicity of B. thuringiensis toxin, which is commonly used for larval source reduction in Sri Lanka. Furthermore, the differential survival and LC$_{50}$ and LC$_{90}$ values of fresh and brackish water-derived larvae tend to suggest that Ae. aegypti may be able to adapt to salinity in its environment. The results indicate that Ae. aegypti derived from brackish water habitats may be better able to withstand the toxicity of BACTIVEC® in more brackish waters. A previous study on the effectiveness of two commercial formulations of B. thuringiensis toxin for the control of salinity-tolerant malaria vector Anopheles aquasalis at different salt concentrations revealed an increase in the LC$_{50}$ for B. thuringiensis at higher salt concentrations [26]. Our results suggest that crystal formulation of B. thuringiensis toxin sprayed in the more brackish water pre-imaginal development sites of Ae. aegypti as a source reduction strategy in coastal zones may be less effective than in fresh water habitats. The nature of the interaction between salinity and the tested commercial formulation of
B. thuringiensis toxin (BACTIVEC®) is not known. This could be a consequence of the effect of salinity on the toxin formulation itself or to an effect on mosquito physiology that modulates the response to the toxin. Further investigations are needed to clarify the two possibilities.

Many countries have used B. thuringiensis for malaria and dengue vector control with varying degrees of success [27–30]. However, the effect of salinity on the activity of B. thuringiensis toxin against larvae of other mosquito vector species e.g. Ae. albopictus and An. sundaicus s.l. in brackish and saline water also merits detailed investigation. It is possible that other commercial formulations of B. thuringiensis toxin may behave differently if the effect is due to salt on the toxin formulation itself and not larval physiology.

Conclusion

The present study identifies several mosquito vectors of human disease that undergo pre-imaginal development in brackish or saline waters in coastal areas of the Jaffna district in northern Sri Lanka. Salt has a small but significant negative impact on the toxicity of B. thuringiensis to Ae. aegypti larvae at salinity levels where larvae are found in the environment, and this has to be taken into consideration for its use as a larvicide.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

SNS, RR and MS conceived the study. PJJ and SK performed field studies, TT, Jaffna, Jaffna, Sri Lanka. 3Department of Pathology, Faculty of Medicine, Walter Reed Biosystematics Unit, 193, Geological Survey of Canada; 2012. http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf. 5. Surendran SN, Kannathasan S, Kajatheepan A, Jude PJ: Chloroquine resistance in Jaffna district, northern Sri Lanka. Malar J 2007, 35:249–252.

References

1. World Health Organization: Dengue Guidelines for Diagnosis, Treatment, Prevention and Control. 2011. WHO/HTM/NTD/IBN/2009.1 http://whqlibdoc.w HO.int/publications/2009/9789241547871_eng.pdf (Accessed July 12, 2011).

2. Weaver SC, Reisen WK: Present and future arboviral threats. Antiviral Res 2010, 85:328–345.

3. Cavirini F, Gaiban P, Pierro AM, Rossini G, Landini MP, Sambri V: Chikungunya: an emerging and spreading arthropod-borne viral disease. J Infect Dev Ctries 2009, 3:744–752.

4. Epidemiology Unit: Ministry of Healthcare and Indigenous Medicine, Sri Lanka. 2012. http://www.epid.gov.lk/web/index.php?option=com_cms&caseand deaths&Itemid=4448&lang=en=

5. Surendran SN, Kannathasan S, Kajatheepan A, Jude PJ: Chikungunya-type fever outbreak: some aspects related to this new epidemic in Jaffna district, northern Sri Lanka. Malar J 2007, 35:249–252.

6. Anti-Malaria Campaign, Sri Lanka. 2012. http://www.malarialcampaign.gov.lk/ Presentation/Home.aspx.

7. Anti-Filarial Campaign, Sri Lanka. 2012. http://203.94.76.60/department/dgfilaria.htm.

8. Epidemiology Unit: Ministry of Healthcare and Indigenous Medicine, Sri Lanka. 2012. http://www.epid.gov.lk/web/index.php?option=com_comcontent&view=art pe&catid=456&lang=en.

9. Boivert M: Utilization of Bacillus thuringiensis var. israelensis (Bti)-based formulations for the biological control of mosquitoes in Canada. In Pacific Rim Conference on the Biotechnology of Bacillus thuringiensis and its Environmental Impact. 6th edition. Edited by Côté JC, Orlos IS, Schwartz JL. Vincent C. 2005:87–93.

10. Ramasamy R, Surendran SN, Jude PJ, Dhanshri S, Vinobaba M: Larval development of Aedes aegypti and Aedes albopictus in peri-urban brackish water and its implications for transmission of arboviral diseases. PLoS Negl Trop Dis 2011, 5(11):e1369.

11. Surendran SN, Jude PJ, Thabothiny V, Raveendran S, Ramasamy R: Pre-imaginal development of Aedes aegypti in brackish and fresh water urban domestic wells in Sri Lanka. J Vect Ecol 2012, in print.

12. Ramasamy R, Surendran SN. Global climate change and its potential impact on disease transmission by salinity-tolerant mosquito vectors in coastal zones. Front Physiol (Systems Biology) 2012; 3:198. doi:10.3389/fphys.2012.00198.

13. Rajasooriyar LD, Mathavan V, Dharmagunawardene HA, Nandakumar V: Groundwater quality in the Valilagram region of the Jaffna Peninsula, Sri Lanka. In Sustainable Groundwater Development. Edited by Hiscock KM, Rivett MO, Davison RM. London: Special publications 193, Geological Society; 2002:181–197.

14. Ameenisinghe FP. A guide to the identification of the anopheline mosquitoes (Diptera: Culicidae) of Sri Lanka. I. Adult females. Cey J Sci (Bio Sci) 1990, 21:1–16.

15. Ratanarithikul R, Harbach RE, Panthusiri P, Jones JW, Coleman RE: Illustrated keys to the mosquitoes of Thailand. II. Genera Culex and Lutzia. Southeast Asian J Trop Med Public Health 2005, 36:Suppl 2:1–97.

16. Rueda LM: Pictorial keys to the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. (Zootaxa, 589) Auckland, New Zealand: Magnolia Press; 2004:42–49.

17. Jude PJ, Dhanshri S, Vinobaba M, Surendran SN, Ramasamy R: Anopheloculicicories breeding in brackish waters in Sri Lanka and implications for malaria control. Malar J 2010, 9:106.

18. Vythilingam I, Tan SB, Krishnasamy M: Susceptibility of Culex sitiens Japanese encephalitis virus in peninsula Malaysia. Trop Med Int Health 2002, 7:539–540.

19. Walter Reed Biosystematics Unit. 2012. http://wbvu命令/command_aon. M2Q.html.

20. Peiris JSM, Ameenisinghe FP, Ratnayake CB, Karunaweera ND, Surendran SN: Studies on prevalence of anopheline species and community perception of malaria in Jaffna district, Sri Lanka. J Vector Borne Dis 2012, 49:331–239.

21. Walter Reed Biosystematics Unit: Walter Reed Biosystematics Unit. 2012. http://wbvu命令/generapages/lutzta.htm.

22. Osborn FFR, Heneira MJ, Gomez CJ, Salazar A: Comparison of two commercial formulation of Bacillus thuringiensis var. israelensis for the control of Anopheles aquasalis (Diptera: Culicidae) at three salt concentrations, Mem Inst Oswaldo Cruz, Rio de Janeiro 2007, 102:659–72.

23. Nyarango PM, Gebremeskel T, Melseabtu G: A steep decline of malaria morbidity and mortality trends in Eritrea between 2000 and 2004: the effect of combination of control methods. Malar J 2006, 5:33.
28. Lee YW, Zairi J: Field evaluation of *Bacillus thuringiensis* H-14 against *Aedes* mosquitoes. *Trop Biomed* 2006, 23:37–44.

29. Russell TL, Brown MD, Purdie DM: Efficacy of VectoBac (*Bacillus thuringiensis* variety *israelensis*) formulations for mosquito control in Australia. *J Econ Entomol* 2003, 96:1786–1791.

30. Lee VJ, Ow S, Heah H, Tan MY, Lam P, Ng LC, Lam-Phua SG, Iman A, Seet B: Elimination of malaria risk through integrated combination strategies in a tropical military training island. *Am J Trop Med Hyg* 2010, 82(6):1024–1029.

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