Interceptions of *Aedes aegypti* and *Aedes albopictus* in the port of Darwin, NT, Australia, 25 January and 5 February 2010

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Abstract

There were 2 exotic mosquito interceptions at a port facility in Darwin NT on the 25 January and 5 February 2010. Aedes aegypti larvae and pupae were collected live from a tank container offloaded from a vessel from Timor Lesté. The receptacle and all other receptacles in the port facility were treated with residual insecticide and the area fogged to kill any possible importations of adult mosquitoes.

Follow up surveillance collected Aedes albopictus males in a Biogents trap near the first importation site. Further surveys and elimination measures were undertaken. There have been no further detections of any adults or larvae of these 2 species.

The importations have revealed a new mode of transport of exotic mosquitoes into the NT and indicate further inspection, surveillance and treatment requirements to maintain the NT free of dengue mosquitoes.

Keywords: Aedes aegypti, Aedes albopictus, mosquito, eliminate, dengue vectors.

Introduction

The Northern Territory (NT) is one of the very few regions of the tropical world that has a history of the presence of either of the dengue vectors, *Aedes aegypti* and *Aedes albopictus* and is now free of these vectors. However, the Darwin port areas are particularly vulnerable to the importation of exotic mosquito vectors of dengue originating from overseas ports, with numerous recorded incidents of risk importations,[1,4] with the last recorded in 2007.[5]

Recently, there have been 2 successive exotic mosquito interceptions at the Perkins port facility in Darwin on the 25 January and 5 February 2010. The responses to both of these interception events have been guided by the protocols outlined by the National Arbovirus and Malaria Advisory Committee (NAMAC).[6] This report describes the interceptions and the responses to them.

Detection, Elimination and Surveillance

Detection 1 – Aedes aegypti

On the 25 January 2010, the Australian Quarantine Inspection Services (AQIS) collected live *Ae. aegypti* larvae and pupae from cargo on the *M.V. Kathryn Bay* that routinely voyages between Darwin and Singapore via Dili. The cargo in question was offloaded onto Perkins international wharf in Darwin one week prior to the detection. The mosquitoes were detected in a water holding section of an access hatch (Figure. 1) on top of a large fuel tank (Figure. 2), which was deck cargo on the vessel. Medical Entomology (ME) of the NT Department of Health and Families (DHF) assessment was that the previously laid and recently flooded mosquito eggs had probably hatched soon after the vessel arrived in Darwin. The presence of pupal skins indicated that live adults had possibly flown from the cargo.

Figure 1: Non draining access hatch

Sample Identification – Aedes aegypti

After initial identification as *Ae. aegypti*, AQIS forwarded the juvenile mosquitoes to ME for confirmation on the day of collection. The sample was immediately confirmed as *Ae. aegypti,* with 53 x 4th larval instars, 2 x 3rd larval instars and 3 x pupae. All of the pupae were males and 2 were well developed with scales visible through the pupal skin. Larvae and pupae were identified using taxonomic keys.[7] The AQIS officer who collected the sample indicated that a number of pupal skins were present, but were not collected.

Elimination and Survey Procedures – Aedes aegypti

On the same day of the detection, the inspecting AQIS officer and the ME exotic vector surveillance officer treated all the inside surfaces of the tank-container access hatches with a chlorine/detergent mix (~12% active chlorine) applied by a pump sprayer.[8] Perkins personnel had already applied chlorine to the hatch after the sample collection, but it was not certain that all inner surfaces of the hatch had been treated. A residual spray of the synthetic pyrethroid lambda-cyhalothrin was also applied to the area behind the Perkins Quarantine wash down bay by pressure spray. This was where the water recycling system, including retention pits and tanks is located and was the closest potential breeding and harbourage site for any adult mosquitoes that may have emerged from the access hatch breeding site.

On the evening of the detection, ME conducted an Ultra Low Volume (ULV) fogging application of bioresmethrin to the Perkins international shipping area and the general Perkins shipping and engineering yards, together with the adjacent area to the south at the Frances Bay Marine premises (Figure. 3). The wind directed the fog to the east towards the sea. Approval for fogging was given by the Chief Health Officer (CHO). All Perkins personnel were requested to vacate areas potentially exposed to the fog.

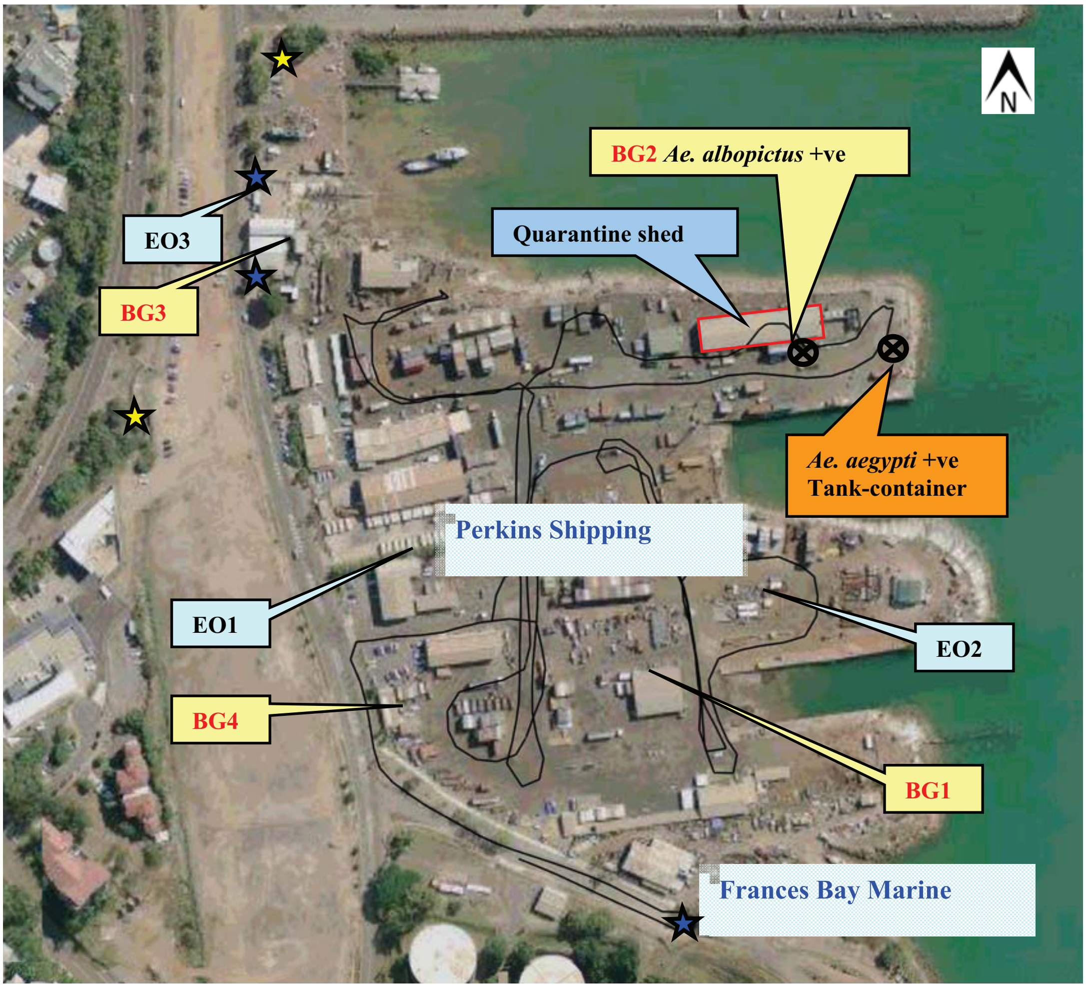
Figure 2. ***Ae. aegypti*** **positive tank-container, Perkins shipping 25 January 2010**

The positive tank-container was 1 of 3 tanks transported on the *M.V. Kathryn Bay*. The 2 other tank-containers were located within the Perkins yard on 27 January. One of these was identical to the positive tank-container, but did not have water pooling in the hatch section. The inside surfaces of the dry hatches were sprayed with a chlorine/detergent mix to kill any possible eggs present. The remaining tank-container did not have access hatches located on top of the tank.

On 3 February, a receptacle treatment round was conducted by applying alpha-cypermethrin (synthetic pyrethroid) by pressure sprayer to all actual and possible water holding receptacles within the entire Perkins yard. This procedure included sampling receptacles for larval mosquitoes. Recent trials testing alpha-cypermethrin and lambda-cyhalothrin found that both successfully prevented mosquito breeding in receptacles for up to at least 10 weeks, with evidence that alpha-cypermethrin provided better knock down for harbouring adults than lambda-cyhalothrin.[9] In the NT, the Australian Pesticides and Veterinary Medicines Authority provided an interpretation that alpha-cypermethrin was able to be used in accordance with the label rates for the treatment of surfaces to kill adult mosquitoes

Prior to this interception, ME and AQIS had conducted joint receptacle surveys on 13 November 2009 for exotic mosquitoes at risk locations within the 400m quarantine zone in the Darwin port area. This incorporated the treatment of actual and potential breeding receptacles with S-methoprene pellets to prevent the emergence of adult mosquitoes. These applications of S-methoprene were assessed as still active (up to and including the first week of February 2010), when reared *Aedes tremulus* larvae from a marked-as-treated tyre either perished upon pupation or did not survive to the pupal stage. Many earthmoving truck tyres were identified as potential breeding sites during this survey and removal or drilling on multiple surfaces to allow for complete drainage was recommended.

Figure 3: Exotic mosquito interception locations 25 January - 5 February; enhanced adult surveillance locations; fog route 25 January, and sites of human landing collections 8 February 2010



⊗ Interception	EO	Standard Ovitrap (1,2,3)	Human Landing Collections	
Fog route	BG	CO_2 baited BG trap (1,2,3,4)	☆	Endemic mosquitoes collected
			★	No mosquitoes collected

Enhanced Surveillance Response – Aedes aegypti

Ovitraps

ME and AQIS constantly maintain 8 ovitraps (egg traps) within Perkins and the adjacent premises of Frances Bay Marine. AQIS also has a sentinel tyre trap located in the Quarantine shed at Perkins.

On 28 January 2010, 3 extra ovitraps were set and serviced weekly for 4 weeks (Figure. 3).

Adult traps

On 28 January 2010, 4 Biogents® sentinel (BG) traps were deployed. (Figure. 3). The BG traps were baited with CO_2 gas delivered through a regulator attached to a D size gas bottle and these were run continuously for 1 week. The catch bags were collected and reset after ~24 hrs for weekdays and around 72 hrs over the weekend. After the initial week, BG catch bags were collected once per week for 3 more weeks until 5 March.

Detection 2 – Aedes albopictus

A collection of 3 *Aedes albopictus* males was made on 5 February in one of the BG traps set for the detection of additional *Ae. aegypti*. One male was positively identified using a taxonomic key,[7] while the remaining 2 were probably *Ae. albopictus* with considerable scale damage. The 2 rubbed males have been sent for DNA analysis.

The positive trap was located in the Quarantine shed on the international wharf at Perkins, and is adjacent to where overseas vessels berth (Figure 3, BG trap 2). The last vessel to have berthed there was the *M.V. Arafura Endeavour* on 29 January. Since this vessel voyages along the same route as the *M.V. Kathryn Bay*, the *Ae. albopictus* originated either from Singapore or Timor Lesté. It is possible that the *Ae. albopictus* adults could have harboured in shipping containers or on the vessel. On docking or opening of the shipping container for inspection, the mosquitoes could have dispersed to the BG trap (Figure 4).

Figure 4. Quarantine shed with opened shipping container and baggage being inspected. BG trap 2 (circled).

Elimination and Survey Procedures – Aedes albopictus

On the same day as the *Ae. albopictus* detection (5 February), a receptacle survey was conducted within the vicinity of the international wharf to detect any new, or previously overlooked potential mosquito breeding sites. Potential sites were located in the wash down bay channel and sump, and the wash down bay recycled water holding tanks. These areas were treated with alpha-cypermethrin and S-methoprene briquettes. It was noted during this survey that the residual pyrethroid (alpha-cypermethrin) treatment for the previous *Ae. aegypti* interception to earthmoving tyres near the international area contained numerous *Culex quinquefasciatus* mosquitoes dead on the water, indicating good knock down by the insecticide for harbouring adult mosquitoes.

The wash down bay area on the international wharf was targeted for further scrutiny a few days later. This site had water pooling in a steel covered drain channel. On Monday 8 February, the steel covers were removed with a forklift to reveal that the drainage channels were partially blocked with damp silt, about 5 cm deep. The previous pooled water observed on 5 February was no longer present and no larvae or adults were present in the drain. The silt was removed by Perkins the same day and was disposed of according to quarantine procedures. The only remaining water was located in the sump at the end of the drain channels, which then lead to the water recycling system.

The water recycling system behind the wash down bay was assessed in terms of chlorination procedures and system flow dynamics. Some overflow pipes from the holding tanks needed sealing and screening to prevent adult mosquito entry. The access pits also need screening, since the water returning into the system via these pits may sit for extended periods with no further chlorine treatment.

The Perkins yard and Frances Bay Marine premises were fogged on the evening of the *Ae. albopictus* detection.

Additional receptacle mosquito breeding surveys were conducted between 8 and 17 February to the north and south of Perkins, including the Fisherman's Wharf, the 'Duck Ponds' marina, Pearl Marine Engineering, and Frances Bay Marine. The remaining tank-containers from the *M.V Arafura Endeavour* were located, with 3 of these reported to be on the vessel back to Singapore and the remainder held within the Perkins yard. These were inspected and were not holding water. This was due to the inclusion of drainage holes in the design of the access hatches, some of which do not have lids. However, it was noted that these drainage holes were capable of being blocked by accumulations of soil, grass and leaves.

Enhanced Surveillance Response – Aedes albopictus

Ovitraps
The 3 additional ovitraps that were set on 28 January (Figure 3) were continued for an

additional week until 5 March. Routinely placed ovitraps continue to be operated as normal.

Adult traps
Four BG traps were operated continuously for 1 week from 5 February and serviced daily. After 12 February, 2 BG traps were operational for 4 days continuously per week for 4 weeks for the surveillance of further exotic adult mosquitoes.

Human landing collections
Adult mosquito catches using human bait subjects for landing collections were conducted at 5 locations in the late afternoon on 8 February. The collection sites were on the western perimeter of Perkins, Frances Bay Marine, Fisherman's pontoon, and west on the escarpment leading to Darwin City (Figure 3). Collection sites were amongst dense vegetation or in harbourage areas in close proximity to areas of vegetation.

Results

Aedes aegypti

No *Ae. aegypti* adults or larvae were recovered from any of the surveillance methods used after the first interception on 25 January. However, adults specimens of *Aedes katherinensis*, an NT endemic mosquito not recorded in Darwin city for 30 years, were collected at Perkins engineering in BG trap 3 on 1 February, with larvae found in a vehicle tyre in an area ~ 400m north of Perkins on 11 February.

Aedes albopictus

No further *Ae. albopictus* adults or larvae were collected subsequent to the detection on 5 February.

Discussion

It does not appear to be a deliberate design to make the lower section of the access hatches 'water tight', and on inspection of numerous other tank-containers, the incorporation of drainage holes in the design of the lower component of the hatch is common. Any tanks having the water holding design need drainage holes, to allow for complete drainage of any water or spilled fuel.

The *Ae. aegypti* importation within the tank-container access hatch was assessed as a low risk importation, because of the developmental stages present, the small numbers, and the sex ratios, with possibly only 3 to 5 adults emerging and with a high probability that the emergences were all males. In general, with *Aedes* receptacle breeding mosquitoes, males emerge first from any single breeding cohort. The collection of mostly 4th instars, with only a few male pupae supports an assessment that the adult emergences had only just begun when the breeding site was detected.

The risk level for the *Ae. albopictus* interception was considered to be higher than that for *Ae. aegypti*, but only at a moderate level, because the adult stage was involved, and the fact that the source of the adults and the potential numbers of females involved, if any, has not been clearly established.

The most probable explanation for their arrival is that there was a water-holding receptacle on or offloaded as cargo from the *M.V. Arafura Endeavour,* which contained larvae and pupae. A few males escaped, while any remaining larvae and pupae were probably inadvertently destroyed or left on the vessel when it departed.

The alternative explanation of a few harbouring adults on board the vessel or in an offloaded shipping container dispersing to the BG is considered less likely, as the adults were all males. Harbouring adults in normal circumstances would be expected to be mainly females.

The results of the surveillance following the elimination responses indicate that the probability of an establishment of either *Ae. aegypti* or *Ae. albopictus* in Darwin port from these interceptions is now very low.

This is the first time BG traps baited with CO_2 have been deployed by ME and AQIS in surveillance for risk importation events in Darwin port facilities. The current routine adult trap (a modified Encephalitis Virus Surveillance trap) is located ~270m from the unloading area of the international wharf. With the recent addition of the BG baited CO_2 traps as a routine monitoring method at locations in close proximity to the unloading and inspection areas of Darwin port, the probability of detections of exotic adult mosquitoes is now likely to increase. The detection of the *Ae. albopictus* males and of the endemic *Ae. katherinensis* demonstrates the effectiveness of the CO_2 baited BG traps.

Recommendations

Tank –container access hatches
Any tanks having the water holding design need drainage holes or a larger gap at the base of the hatch flush with the tank surface to allow for complete drainage of any water or spilt fuel.

Recycled water storage tanks
The over flow pipes from the holding tanks behind the wash down bay at Perkins international wharf should be screened and any gaps in pipe connections sealed. The inspection hatches on top of the tanks need to be repaired or replaced to ensure they are completely sealed.

Water recycling pits
Although the water in the main holding tank is kept at 5 ppm of chlorine, it is possible for chlorine in the water recovery pits to be diluted by rainwater runoff or be reduced to low levels when there are periods of infrequent use. Chlorine could then fall to a non-lethal level for larval mosquitoes.

The pits associated with the recycled water tanks behind the wash down bay need to be appropriately screened to prevent adult mosquito entry.

Wash down bay
The drainage channels that contained silt need to be routinely cleared to prevent water pooling. The bottom of the channel should be inspected and re-concreted if necessary so that a slope is created, which will allow water to effectively drain out of the channels and into the sump.

Wash down bay sump
Water pools in the wash down bay sump and, because the outlet pipe is ~ 5 cm above the level of the sump, can also pool for a distance along the drain channels. The channels and sump should be re-concreted to allow for more complete drainage of the water, or the outlet pipe could be lowered and screened.

Routine adult mosquito surveillance
At least 1 CO_2 baited BG trap should be maintained in the Perkins quarantine area for at least a 4 day collection period each week. There should be consideration to run this trap over a 7 day period with twice weekly catch collections. Regularly running a BG trap in this area increases the probability for exotic mosquito detection in close proximity to docking, offloading and inspection areas receiving overseas cargo vessels or any vessels originating from north Queensland where *Ae. aegypti* or *Ae. albopictus* occur.

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Salt marsh mosquito larval control in Leanyer coastal wetland, Northern Territory

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Abstract

A coastal wetland with important larval habitats for Aedes vigilax (Skuse), the northern salt marsh mosquito is located adjacent to the northern suburbs of Darwin. This species is a vector for Ross River virus and Barmah Forest virus, as well as an appreciable human pest. To improve aerial larval control, we identified the most important vegetation categories and climatic/seasonal aspects associated with aerial control operations in this wetland after inundation with tide, rain and tide and rain combined.

The analyses showed that the Schoenoplectus/ mangrove areas require most of the control after inundation by tide only (30.1%), and also extensive control when tides and rain are coinciding (18.2%). Tide-affected reticulate vegetation requires extensive control after inundation by rain only (44.7%), and when tide and rain inundation coincide (38.0%). The analyses further showed that most of the control needs to be carried out between September and January, with a control peak in November and December.

To maximise the efficiency of aerial salt marsh mosquito control operations in northern Australia, aerial control efforts should concentrate on Schoenoplectus/mangrove and tide-affected reticulate areas, especially between September and January.

Keywords; mosquito, mosquito control, Aedes vigilax, Schoenoplectus/mangrove, wetlands.

Introduction

The Darwin area experiences a monsoon climate, with most rainfall occurring between November and April. A number of the northern Darwin residential suburbs are located close to an extensive coastal reed and upper mangrove wetland, which experiences seasonal tidal inundation. This wetland provides suitable breeding conditions for the northern salt marsh mosquito *Aedes vigilax*.[1] This mosquito species is not only a human pest species[2,3] but is also a major vector for Ross River virus and Barmah Forest virus in the Northern Territory (NT).[4,5] Thus, control of *Ae. vigilax* is of great importance for disease prevention and nuisance reduction in the NT.

Medical Entomology of the Department of Health and Families conducts integrated mosquito control for *Ae. vigilax* breeding in this wetland.[6] This paper outlines the vegetation categories requiring most of the control effort for *Ae. vigilax* larvae, and shows in which months most of the control needs to be carried out. By identifying the most important