Mosquito Bites
In the Asia Pacific Region

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Murray Valley Encephalitis Virus Detection using Honeybait Cards in the Northern Territory in 2013

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**Background**

Murray Valley encephalitis virus (MVEV) and Kunjin virus (KUNV) are endemic flaviviruses in Australia (Russell 1998; Russell and Dwyer 2000). In the Northern Territory (NT), the principal vector for both viruses is Culex annulirostris (Russell 1998; Russell and Dwyer 2000), with herons and egrets being known hosts (Russell and Dwyer 2000). Although not very common, Murray Valley encephalitis is a serious disease and can potentially be fatal (Bennett 1976; Mackenzie et al. 1993, Burrow et al. 1998). In the NT, 34 MVE and 12 KUN virus disease cases have been reported since 1974 (Medical Entomology annual report 2012/13).

The sentinel chicken program in the NT is part of a national program involving the NT, WA, NSW and VIC and is designed to detect flavivirus activity. The current NT program commenced in 1992, and sentinel chicken flocks are maintained, bled and analysed for flavivirus in a combined program between Medical Entomology of the Department of Health (DoH), the virology laboratories of the Department of Primary Industry and Fisheries (DPIF) and volunteers. This program enables the DoH to issue timely MVE media warnings to the public.

However, flavivirus surveillance in remote areas can be logistically difficult. To aid the situation, a new sugar baited surveillance system (honey bait traps) was recently developed in Queensland to detect alpha and flaviviruses, with traps able to remain in the field for extended periods (Hall-Mendelin et al. 2010; Ritchie et al. 2013). The honey bait traps are CO\textsubscript{2} baited passive box traps containing honey-soaked nucleic acid preservation cards, which are available for the mosquitoes to feed on prior to the cards being tested for virus. (Hall-Mendelin et al. 2010; van den Hurk et al. 2007).

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The first trial of the new surveillance system was conducted by Qld Health in liaison with NT DoH and DPIF in 2012 (van den Hurk et al. 2013). In the Darwin area, two honey bait traps were deployed in parallel with sentinel chickens. During the trial, Kunjin, Ross River and Barmah Forest viruses were detected but not MVE virus. No sentinel chickens tested positive for MVEV or KUNV at the two NT sites that year, most likely due to the virus circulating at very low levels.

In 2013, the NT DoH re-deployed two honey bait traps in parallel with sentinel chickens to further test the ability of the new surveillance system to detect MVEV. This report describes the methods and results of the 2013 NT trial.

**Methods**

Between December 2012 and June 2013, one honey bait trap, as described in Hall-Mendelin et al. 2010, was set in Leanyer (Darwin), with a second trap set at Beatrice Hill Farm (BHF), located 60km east of Darwin (Figures 1 and 2). BHF is surrounded by
extensive freshwater wetlands, while the Leanyer site is adjacent to a salt marsh, brackish and freshwater swamps. Honey bait cards were collected fortnightly and tested for MVEV and KUNV at the Berrimah Veterinary Laboratories (BVL) in Darwin, (Pyke et al. 2004). Mosquitoes were collected weekly and identified to species level using taxonomic keys at the Medical Entomology Laboratory.

In parallel, the sentinel chickens in Leanyer (12 chickens) and BHF (10 chickens) were bled monthly until June 2013, with chickens at BHF first bled in late November and in Leanyer in early December 2012. Blood samples were tested for MVEV and KUNV at BVL.

Results

A total of 72 and 78 honey bait cards were tested for MVEV and KUNV from BHF and Leanyer respectively. All cards tested negative, except for cards collected at BHF on 18 April 2013, testing positive for MVEV, with a Ct score of 38.2.

A total of 79776 mosquitoes were collected between 10 January and 19 June 2013 at BHF, consisting of Coquillettidia xanthogaster (40.46%), Culex annulirostris (30.69%), Mansonla uniformis (21.02%) and other species (7.84%).

A total of 23210 mosquitoes were collected between 12 December and 26 June 2013 at Leanyer, consisting of Cx. annulirostris (51.09%), Aedes vigilax (24.66%), Cx. sitiens (11.26%) and other species (12.96%).

A total of 475 sentinel chickens were tested for flavivirus in the NT, with 71 and 84 chickens tested at BHF and Leanyer respectively. No chickens seroconverted to MVEV or KUNV.
Discussion

The new flavivirus surveillance system has already been proven to be an effective tool for detecting some alpha and flaviviruses, with RRV, BFV and KUNV detected in Qld and the NT. However, no MVEV was detected in the initial trial in 2012.

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Honey bait cards with a threshold cycle number (Ct) of less than 45 are considered positive for flavivirus (van den Hurk et al. 2013; Hall et al. 2011). The first detection of MVEV from honey bait cards in the NT in April 2013, with a Ct score of 38.2 shows that the system is capable of detecting MVEV activity. In addition, MVEV was detected using honey bait cards, when no sentinel chickens seroconverted to MVEV in the NT. This shows the sensitivity of the new system and suggests that it is capable of detecting virus circulating in very low levels. Furthermore, the fact that a relatively high proportion of the total mosquitoes caught in the honey bait traps were *Culex annulirostris*, the principal vector for MVEV, implies the suitability of the new system for MVEV surveillance, and suggests that it might be feasible to replace previously deployed flavivirus surveillance tools.

However, during the 2013 NT trial, technical issues frequently occurred, leading to trap failure and at times low mosquito counts in the honey bait traps. Issues most frequently encountered included reduced gas flow to well below the set 250 ml/min, and failure of the actual gas flow timer. Therefore, it is suggested that these technical issues be addressed prior to honey bait traps being considered for deployment in remote areas. In addition, costs associated with the new system should be evaluated to assess the cost effectiveness before replacement of conventional flavivirus surveillance system is considered.

References


Medical Entomology annual report 2012/13


