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Extensive public health initiatives drive the elimination of Aedes aegypti from a town in regional Queensland: a case study from Gin Gin, Australia.

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Extensive public health initiatives drive the elimination of *Aedes aegypti* from a town in regional Queensland: a case study from Gin Gin, Australia.

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Key words: Elimination, Aedes aegypti, Public Health, Community Engagement, Rainwater Tank, Population Genetics

Abstract

Aedes aegypti is the primary vector of exotic arboviruses (dengue, chikungunya and Zika) in Australia. Once established across much of Australia, this mosquito species remains prevalent in central and northern Queensland. In 2011, *Ae. aegypti* was re-discovered in the town of Gin Gin, Queensland, by health authorities during routine larval surveillance. This town is situated on a major highway that provides a distribution pathway into the highly vulnerable and populous region of the state where the species was once common. Following the detection, larval habitat and adult control activities were conducted as a public health intervention to suppress the *Ae. aegypti* population and reduce the risk of exotic disease transmission. Importantly, genetic analysis revealed a homogenous cluster and small effective population vulnerable to elimination. By 2015, the population had expanded throughout the centre of the town. In response, a collaboration between research agencies and local stakeholders activated a council initiative in 2016 that included extensive community engagement, enhanced entomologic surveillance and vector control activities including the targeting of key containers such as unsealed rainwater tanks. Here we describe a model of the public health intervention which successfully reduced the *Ae. aegypti* population below detection thresholds, using source reduction,

insecticides and novel, intensive genetic surveillance methods. This outcome has important implications for future elimination work in small towns in vulnerable regions and reinforces the longstanding benefits of a partnership model for public health-based interventions for invasive urban mosquito species.

Introduction

Aedes aegypti (Linnaeus) (Diptera: Culicidae) is the primary vector of dengue fever, chikungunya and Zika on mainland Australia. Globally, *Ae. aegypti* is responsible for the rapid re-emergence and spread of viral diseases over the past 40 years (1). Currently, the number of annual global dengue cases is estimated to be 390 million (2), while the Zika epidemic in South America during 2015 was largely attributed to this species (3). *Aedes aegypti* exhibits several characteristics which make it one of the most invasive of all mosquito species. These include a lifecycle highly adapted to urban environments including; a penchant for human blood with multiple feedings per gonotrophic cycle, desiccation resistant eggs that can survive dry and winter conditions, and the utilization of domestic containers as larval habitat (4). Historically, these traits have enabled the species to spread widely within Australia, causing epidemics of dengue fever which have shaped public health policy (5).

Aedes aegypti is postulated to have been introduced into Australia around the time of British colonization, primarily through water storage on large sailing ships (6). By the early 1900s it had spread throughout the eastern seaboard as far south as the Victorian border (7). During the early part of the 20th century, *Ae. aegypti* was responsible for large epidemics of dengue fever in Queensland, with some affecting up to 90% of populations in major urban centres such as Brisbane (7). With a rapid increase in dengue prevalence worldwide since the 1980s, Queensland observed a resurgence of the disease in north Queensland (8). The artificial introduction of the *Wolbachia* symbiont into *Ae. aegypti* populations in 2010 (9) has subsequently reduced the threat of autochthonous dengue outbreaks in northern Queensland (10).

Traditional *Ae. aegypti* management focuses on the removal of larval habitat and the application of insecticides to either control adults or larvae (11). In Australia, the distribution of *Ae. aegypti* has retracted into central Queensland following the adoption of reticulated water and intensive post-World War 2 public health interventions that removed rainwater tanks (a permanent source of larval habitat)(5, 12). Health authorities targeting larval habitat in the state's capital, Brisbane, led to the elimination of *Ae. aegypti* around 1957 and cessation of local dengue transmission since 1947 (5). More recently, the elimination of incursions of *Ae. aegypti* populations from Queensland into the Northern Territory (Tennant Creek and Groote Eylandt) were undertaken through effective community education, larval source reduction and targeted insecticide treatments and monitoring programs (13, 14). These same processes form the core strategy developed by the Queensland government to manage dengue transmission (11).

Central and southern Queensland regions remain vulnerable to exotic disease outbreaks where the wild-type *Ae. aegypti* (no *Wolbachia* infection) vector is abundant and interacts with infective travellers. During mid-2019, Rockhampton recorded the first dengue outbreak in over 60 years (15, 16). It is unknown whether areas outside north Queensland are suitable for *Wolbachia* introduction (17, 18). Thus, traditional methods of mosquito control remain the strategy to reduce the risk of disease transmission by health authorities in southern Queensland regions (11). South East Queensland (SEQ) is highly vulnerable to the establishment of *Ae. aegypti* (19) and receives a high proportion of the annual numbers of viraemic travellers to Queensland (20). Developing regional capacity and capability alongside innovative strategies to eliminate invasive mosquito populations is particularly important when minimizing the risk of exotic disease transmission in large urban centres.

In 2011 *Ae. aegypti* was re-discovered in the small town of Gin Gin after 25 years through house-to-house surveys by state health authorities. The species was not identified in small surveys in 1996 and 2006 (Brian Montgomery, Queensland Health, pers. comm.). Here we provide a public health perspective and document the extensive and enhanced entomological surveillance and control

activities required to eliminate *Ae. aegypti* from Gin Gin that serve as a calibration exercise for similar or larger-scale interventions.

Methods

Chronology of intervention and the various annual activities are described below.

Summer 2011-2012. Initial Detection, Surveillance, Control and Community Engagement

Gin Gin (24.9908° S, 151.9500° E) is a small town (1,053 population)(21) in the Wide Bay Burnett region of Queensland, Australia, located on a major highway into the state capital of Brisbane (Figure 1). In Queensland, local governments are responsible for monitoring and enforcing the *Public Health Act 2005* (22) and *Public Health Regulation 2005* (23). Following the detection of *Ae. aegypti* in Gin Gin during 2011, a report was provided to the Bundaberg Regional Council (BRC) by Queensland Health. Recommendations to prevent the regional spread of the species included:

1) Elimination of the Gin Gin *Ae. aegypti* population to reduce the risk of dengue transmission (from viraemic travellers).
2) Surveying premises in which *Ae. aegypti* were detected (at least once a month for five months during summer), to determine whether *Ae. aegypti* was still present.
3) Surveying premises adjacent to those where *Ae. aegypti* were present to ensure these were also free of the species.

BRC conducted routine adult surveillance at and around the premises where *Ae. aegypti* was first collected (Figure 2A). Oviposition traps (ovitraps) and a network of BG Sentinel traps (24)(BGS; Biogents GmbH, Regensburg, Germany) for adults were set at key premises and collected weekly between the 4th and 11th of May and the 4th of May and 25th of October 2011, respectively. A population suppression program was implemented with BRC the lead agency and technical support provided by Queensland Health. Larval surveillance and control was undertaken over a two week

period (30th January to 17th February 2012). Council staff were trained in house-to-house inspection methodologies and larval surveillance techniques prior to an extensive larval

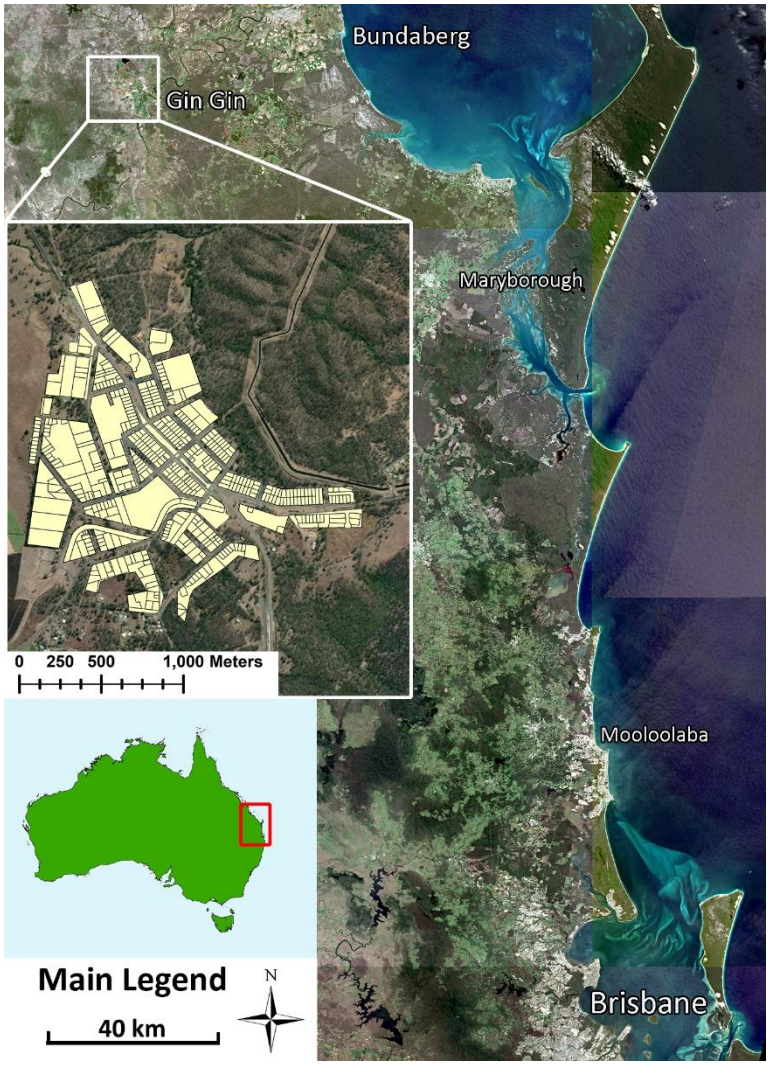

Figure 1. Location of the town of Gin Gin relative to Brisbane and South East Queensland. Location within Australia (bottom left) and Gin Gin town layout including scale (middle left). Map Source: Base Layer assembled from the Open Access Copernicus Australasia Regional Data Hub (38) and Australian map and residential features digitized from public domain cadastre data (37) in ArcGIS.

survey (480 premises). At each premises, verbal consent was obtained to conduct an inspection and if nobody was present the property was re-visited. Premises were scored by the Premise Condition Index (PCI)(25) and all natural and artificial containers holding water were checked for mosquito larvae. A sub-sample (6 to 12 larvae) from each positive larval habitat was placed into 80% ethanol for

118 species identification by microscopy. Adult surveillance was undertaken by BGS traps set fortnightly
119 (10 premises, 22nd March – 14th May 2012) and ovitraps set weekly (14 premises; 21st March – 22nd
120 May 2012).

121 Legal mechanisms in Queensland were activated to enable this intervention. Firstly, an 'Authorised
122 Inspection Program' was implemented under the *Local Government Act 2009* to grant powers of entry
123 to yards (not inside houses) by authorised officers without a resident's consent. Secondly, all chemical
124 treatment was consistent with label recommendations, conducted by or supervised by a licensed Pest
125 Management Technician (PMT) and a *Pest Control Advice* (PCA) provided for each premises when
126 treatment occurred. Larval control activities consisted of the removal and/or insecticide treatment of
127 containers that contain or have the potential to contain *Ae. aegypti*. Prolink Pellets® containing the
128 insect growth regulator (*S*)-methoprene were applied to containers that were either; hard-to-inspect,
129 or were large or could not be emptied or removed (e.g. drain sumps, drums, tyres, tree holes). Prolink
130 ProSand® was applied to leaf axils of bromeliads that retain water. To prevent the emergence of adult
131 mosquitoes from large permanent water sources, Prolink XR Briquets® were used in damaged
132 rainwater tanks or if their screens had been removed. For adult control, two lethal ovitraps (26) were
133 set at each of the positive premises.

134 As part of the 2012 elimination strategy, a community engagement plan was established through
135 targeted awareness campaigns and community engagement strategies including:

136 • a 'Survey to eliminate *Aedes aegypti* to reduce a public health risk' fact sheet,
137 • a media release on the 'Gin Gin *Ae. aegypti* elimination program',
138 • letter for premises that were positive for *Ae. aegypti*,
139 • letter for premises within a 100 m radius of premises that were positive for *Ae. aegypti* and,
140 • an information sheet regarding prevention and control of mosquito breeding in the yard.

141 A full description of 2012 surveillance, control and community engagement methods is documented
142 in S1 Appendix.

2012-2013. Genetic Assessment

A population genetics study was undertaken by BRC to understand the likelihood of *Ae. aegypti* population elimination following removal of the extant population. Samples of larvae (n=39) collected from containers during house-to-house surveys (April 2013) were sent to University of Melbourne (Pest and Environmental Adaptation Research Group)and assessed at the genetic level for population structure using neutral microsatellite markers and compared with other published data on samples from central Queensland (27). Allelic richness calculated from 15 individuals to match the size of the smallest population in the central Queensland study)(A), gene diversity (He), pairwise F_{ST} and inbreeding coefficient (F_{IS}) were estimated using FSTAT 2.9.3.2. Mean effective population size (Ne) was estimated using ONeSamp 1.2. A Bayesian analysis to estimate the number of populations within the sample data was made using STRUCTURE (Version 2) (28). A burn-in length of 100 000 was chosen followed by 250 000 iterations and the simulation was run using the admixture model with allele frequencies uncorrelated among populations. The number of populations within the data (*K*) is estimated by checking the fit of the model for a range of *K* values. *K* values of 1 to 8 were tested with five runs for each value of *K*. We used the method of Evanno *et al.* (29) to estimate the true *K* as applied in STRUCTURE Harvester.

2013-2014. Surveillance Activities

A larval and adult trapping survey was undertaken by Queensland Health (February to April 2014). House-to-house surveys were undertaken at 73 premises, and Gravid *Aedes* Traps (GAT)(30) were placed within six of these for 13 days, serviced after seven days.

2014-2015. Surveillance Activities

A rainwater tank survey on eight rainwater tanks in central Gin Gin premises was performed in December 2014. Tank compliance and a larval sample was collected using the methods of Knox *et al*. (31). Three ovitraps were set around unsealed rainwater tanks for four weeks.

An oviposition study (February - mid-April 2015) was undertaken at nine premises for ten weeks. Four ovitraps and a single GAT containing aged rainwater, a lucerne pellet (32) and water-proof sandpaper (80 grit) used as an oviposition substrate, were placed within the yard of each premises. Traps were serviced fortnightly, eggs counted, and larvae reared to fourth instar for identification to species via microscopy (33).

2015-2016. Surveillance, Experiment, Traditional Control and Community Engagement

A mark-release-recapture (MRR) study within Gin Gin was undertaken in 2016 to understand the distribution and movement of *Ae. aegypti* (34). Forty premises and 58 rainwater tanks were surveyed with the adult trapping methodology and rainwater tank non-compliance survey documented in Trewin *et al.* (34). As part of this study, extensive efforts were made to engage and educate the local community on the risk of *Ae. aegypti* breeding on their premises (S2 Appendix). Community engagement included the formation of a community reference group, town hall meeting, educational flyers and media activities. Homeowners were encouraged to clean-up surplus containers from yards and seal rainwater tanks. Prior to the MRR study, all non-compliant rainwater tanks as defined by regulatory standards (e.g. mesh size aperture to prevent entry or egress of mosquitoes) were sealed with new mesh screens or silicone in rust-related holes. Tanks unable to be sealed were treated with residual insecticides (Prolink XRBriquets®) and residents were encouraged to decommission high risk tanks. Traditional mosquito control was undertaken as part of the risk mitigation strategy including source reduction, residual insecticide treatments of Prolink ProSand® in bromeliads, and Prolink XRBriquets® in larger containers. Indoor residual spraying (35) was offered to residents once the experiment was complete (with two residents and one business opting to have their premises treated). Residents were compensated for their participation through an inexpensive voucher system for redeeming local produce. For 2016 community engagement and risk management and mosquito population suppression plans see S2 Appendix, S3 Appendix and S4 Appendix, respectively.

2017-2018. Surveillance Activities

Records for surveillance by BRC during 2016-2017 were unavailable for analyses. During summer 2017-2018 adult surveys using BGS traps for five weeks across five premises in late summer and classified adults to species by microscopy.

2018-2019. Surveillance Activities

As part of a larger regional population genetics survey, BGS and ovitrapping was undertaken in Gin Gin for ten weeks (February until May 2018), in six houses previously positive to *Ae. aegypti*. A single BGS trap and four ovitraps were placed in the yard of each house and serviced weekly. Concurrent BGS surveillance was undertaken by BRC (five weeks across five premises) in late summer. Larvae (reared to 4th instar) and adults were identified to species via microscopy (33).

2019-2020. Presence-absence surveillance: Rapid Surveillance for Vector Presence Survey

To interrogate the detection threshold indicated by negative records from the previous three summers, a highly sensitive *Ae. aegypti* survey was conducted using an innovative method that links ovitrap samples to molecular diagnostics. Rapid Surveillance for Vector Presence (RSVP) (36) can rapidly detect *Ae. aegypti* nucleic acids by using real-time reverse transcription polymerase chain reaction (RT-PCR) to screen large amounts of genetic material. Large volumes of endemic species can be processed by aggregating egg samples in cohorts (<5000 eggs) that typically are sourced from multiple ovitraps. The sensitivity of RSVP facilitates the efficiency of a regional presence-absence survey of target invasive species over large spatial and temporal scales, particularly when they are expected to be absent or in very low numbers. Since 2017, RSVP has been offered to regional councils in and near SEQ by Queensland Health on a seasonal basis. Premises across the town were selected to ensure all high-risk residential blocks within Gin Gin were sampled for the presence of *Ae. aegypti*. Twenty-one premises were surveyed with a single ovitrap placed within the yard and eggs collected fortnightly for two periods of four weeks (total 8 weeks from February until March 2020).

Mapping

Trapping and larval surveillance results are mapped at the block scale, the spatial unit under which *Ae. aegypti* is optimally targeted due to limited dispersal abilities (34), while also preserving the privacy of individual premises where surveillance was undertaken. All shapefile maps were digitized in ArcGIS by outlining residential features (blocks, roads, highways) and then overlaying public domain cadastre data (37). Base layer imagery of South East Queensland region sourced from the open access Copernicus Australasian Regional Data Hub (38). Trap days is a quantitative measure of the number of traps placed in the environment multiplied by the number of days present when the population was surveyed (39) over a summer season (November until May).

Results

2011-2012. Initial Detection and Elimination Strategy

Aedes aegypti was first detected in a single property in central Gin Gin by a routine Queensland Health house-to-house survey in 2011 (Figure 2A). Mosquito surveillance and suppression activities subsequent to detection revealed *Ae. aegypti* in 2.3% (11/473) of premises throughout central Gin Gin (Figure 2B), representing a modest increase from the 1986 detections (3 premises). During larval surveys, a total of 5,035 wet and 724 dry containers were observed, an average of 12 larval habitat sites per premises. Mosquitoes were present in approximately 40% and 11% of all premises and wet containers, respectively (Table 2). The most prevalent container category positive for *Ae. aegypti* (35%) were garden accoutrements such as plant pots and saucers, birdbaths, buckets and striking pots (S1 Appendix Table 5). Three hundred and forty-seven rainwater tanks (73.4% of premises inspected) were recorded during the survey, with most tanks containing water. Due to difficulty of access, not all tanks were inspected or sampled. Four tanks were positive for mosquito larvae and one was positive for *Ae. aegypti* larvae. Premises previously positive for *Ae. aegypti* remained positive in one of the 14 ovitrap locations and one of three BGS trap locations over the same ten-week period.

Table 1. Prevalence of water bearing containers and number with *Aedes aegypti* juvenile stages in Gin Gin, Australia. Collected during town-wide surveillance activities summer 2011-12.

Category	Total (%)	Wet (%)	*Ae. aegypti* Present (%)
Garden Accoutrement	1,796 (31.2)	1,408 (28.0)	6 (35.3)
Discarded Household Item	460 (8.0)	409 (8.1)	4 (23.5)
Domestic Use Container	259 (4.5)	246 (4.9)	4 (23.5)
Recreational Item	63 (1.1)	62 (1.2)	1 (5.9)
Water Storage	162 (2.8)	157 (3.1)	1 (5.9)
Rubbish	200 (3.5)	159 (3.2)	0
Building Fixture	224 (3.9)	205 (4.1)	0
Natural Habitat	2,248 (39.0	2,044 (40.6)	0
Total	5,759	5,035	17

Figure 2. Detection block 2011 (A) and town-wide larval surveys 2012 (B) of *Aedes aegypti* in Gin Gin. Red indicates blocks where *Aedes aegypti* were detected, green where no *Ae. aegypti* were found to be present, and yellow blocks were not surveyed.

2012-2013. Surveys and Genetic Assessment

Aedes aegypti was present across six blocks in central Gin Gin, collected from yard containers and one rainwater tank (S5 Appendix, S6 Appendix Fig 1). The thirty-nine samples collected showed the lowest degree of allelic richness (i.e. low number of microsatellite alleles adjusted for sample size) and lowest gene diversity of the samples tested, which included a range of locations in central and northern Queensland (Table 3). The inbreeding coefficient for the Gin Gin sample was moderate, but significant (Table 3). Pairwise FST estimates between sample localities revealed significant population differentiation between the samples of *Ae. aegypti* from Gin Gin and all other localities (Table 4). The only samples not significantly differentiated from each other were from Gordonvale and Yorkeys Knob.

Effective population size in Gin Gin, estimated by ONeSamp, was small and similar to most samples from central Queensland (Table 2; mean =19.21, median =19.15, lower 95% CL =13.58, upper 95% CL =28.04). STRUCTURE analysis gave an estimate of six genetic clusters (K) within the complete dataset from Queensland, using both the highest log probability of the data and the ΔK method (Evanno *et al*. (29)) (S5 Appendix Table 2). For K = 6, all locations showed some degree of admixture, but for Gin Gin, admixture was minimal (S5 Appendix Fig 1). Yorkeys Knob and Gordonvale from north Queensland were grouped together; Longreach, Bluff, Duaringa and Emerald were separate clusters, while other locations showed a high degree of admixture (S5 Fig 1).

Table 3. Genetic diversity over seven microsatellite loci for *Aedes aegypti* from eleven locations in Queensland, Australia. Results grouped in regions (north Queensland - *NQL*, and central Queensland - *CQL*) with: sample size (N), allelic richness calculated from 15 individuals (A), gene diversity (He), inbreeding coefficient (F_{IS}), mean effective population size (Ne) and 95% confidence limits (in parentheses) (Table modified from Rašić *et al.* 2014 to include data from Gin Gin).

Name	Code	Latitude	Longitude	N	A	He	F_{IS}	Ne

North QLD								
Yorkeys Knob	1	-16.8094	145.7226	30	3.38	0.544	0.125	43 (22-146)
Gordonvale	2	-17.0966	145.7787	28	3.15	0.494	0.164	
Ingham	3	-18.6533	146.1604	15	3.00	0.519	0.083	15 (10-37)
Central QLD								
Rockhampton	4	-23.3795	150.4995	30	3.13	0.479	0.134	33 (19-101)
Mt Morgan	5	-23.6449	150.3889	34	2.75	0.442	0.108	19 (10-57)
Duaringa	6	-23.7110	149.6710	29	3.19	0.457	0.079	24 (14-72)
Bluff	7	-23.5786	149.0703	32	2.78	0.477	0.054	25 (15-90)
Emerald	8	-23.5162	148.1610	28	3.23	0.426	0.054	65 (33-282)
Capella	9	-23.0837	148.0245	21	2.98	0.461	-0.078	22 (14-63)
Longreach	10	-23.4433	144.2509	28	3.07	0.443	-0.063	27 (17-105)
Gin Gin	11	-24.98946	151.9500	39	2.51	0.329	0.093	19 (14-28)

Table 4. Pairwise FST estimates for eleven samples of *Aedes aegypti* from Queensland, Australia. Bold indicates no significant differentiation (Table modified from Rašić *et al*. 2014 to include data from Gin Gin).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1 Yorkeys Knob	0										
2 Gordonvale	**0.0126**	0									
3 Ingham	0.0420	0.0507	0								
4 Rockhampton	0.0458	0.0272	0.0831	0							
5 Mt Morgan	0.0786	0.0789	0.1191	0.0283	0						
6 Duaringa	0.1117	0.1012	0.1589	0.0423	0.0499	0					
7 Bluff	0.0619	0.0759	0.0683	0.0871	0.1174	0.1541	0				
8 Emerald	0.0548	0.0734	0.0972	0.0527	0.0616	0.1093	0.1333	0			
9 Capella	0.1131	0.1263	0.1172	0.0910	0.1028	0.0611	0.0970	0.1533	0		
10 Longreach	0.0649	0.0708	0.1277	0.0567	0.0651	0.0573	0.1599	0.0735	0.1215	0	

11 Gin Gin	0.0779	0.1134	0.1785	0.1008	0.1455	0.1588	0.1695	0.1119	0.1691	0.1130	0

Summary of Surveillance Activities 2013-2020.

Six positive premises were detected in 2013-2014 (five from larval surveys and from a GAT at a separate premises, across five residential blocks in north-eastern Gin Gin (S6 Appendix Fig 2). Six of eight rainwater tanks inspected were non-compliant with regulations, but not sampled for *Ae. aegypti*.

Larval surveys (2014-2015) suggest *Ae. aegypti* distribution had expanded southward in residential blocks, with five of 10 positive (S6 Appendix Fig 3). The most extensive trapping effort (applying GAT and ovitraps) was undertaken during this season, consisting of 3,150 trap days over a ten-week period (Table 5). During this survey, all non-compliant rainwater tanks were positive (three of three) for *Ae. aegypti*, while three compliant tanks were negative (Table 5). Surveillance (2015-2016) that included a rainwater tank survey for compliance and background *Ae. aegypti* population monitoring during a mark, release, recapture (MRR) experiment is documented in Trewin *et al.* (34). Wild (unmarked) *Ae. aegypti* were collected in twelve of 26 premises (46%), while four of ten rainwater tanks sampled were positive (S6 Appendix Fig 4).

Surveillance activities for 2016-2017 summer season are unavailable, however, this was the first season that BRC reported *Ae. aegypti* to be absent from Gin Gin. Likewise, a five-week BRC survey with five BGS traps in five premises during the 2017-2018 summer season revealed *Ae. aegypti* to be absent (S6 Appendix Fig 5). During the 2018-2019 season both the BRC and the Commonwealth Scientific and Industrial Research Organisation (CSIRO) undertook trapping surveys at ten premises over a ten-week period, representing 1,365 trap days with both BGS and ovitraps, with no *Ae. aegypti* detected (Table 5, S6 Appendix Fig 6).

Enhanced RSVP ovitrap surveillance (2019-2020) did not detect *Ae. aegypti* in any of the blocks where *Ae. aegypti* had been present previously (S6 Appendix Fig 7). A total of 21 premises were surveyed across 13 different blocks for a total of 1,176 trap days across eight weeks (Table 5). A summary of

297 blocks positive to *Ae. aegypti* previous to 2017 suggest the species distribution was primarily in central
298 areas within the town (Figure 4A), while surveillance effort of 3,591 trap days during the period from
299 2017 to 2020 suggests the species is no longer present in high-risk central blocks (Table 5, Fig 4B).

300

301 **Table 5. Summary results of *Aedes aegypti* surveillance activities in the town of Gin Gin, Australia**. Trap days
302 indicate the number of traps placed in the town multiplied by the number of days each sampled over a summer
303 season (November-May).

Summer Season	Survey Type	Premises Surveyed	Positive Premises (%)	Survey Period (days)	Trap Days	Positive Traps (%)
2012-2013	Larval	na	14 (na)	2	na	na
2013-2014	Larval	73	5 (7)	2	na	na
	GAT	6	1 (17)	13	78	1 (17)
2014-2015	Ovitrap	9	4 (44)	70	2,520	6 (3)
	GAT	9	2 (22)	70	630	5 (11)
	Rainwater Tank	7	3 (43)	70	na	na
2015-2016	BG Trap	26	12 (46)	13	455	17 (49)
	GAT	26	2 (8)	13	910	10 (14)
	Rainwater Tank	10	4 (40)	1	na	na
2016-2017	Data Unavailable					
2017-2018	BG Trap	5	0 (0)	35	175	0 (0)
2018-2019	BG Trap	10	0 (0)	105	560	0 (0)
	Ovitrap	6	0 (0)	70	1,680	0 (0)
2019-2020	Ovitrap (RSVP)	21	0 (0)	56	1,176	0 (0)

304

305

Figure 3. Non-compliant rainwater tanks (with anti-mosquito regulations) identified in Gin Gin containing *Aedes aegypti*. Inserts show detailed views of exposed overflows and rusted inflow sieves.

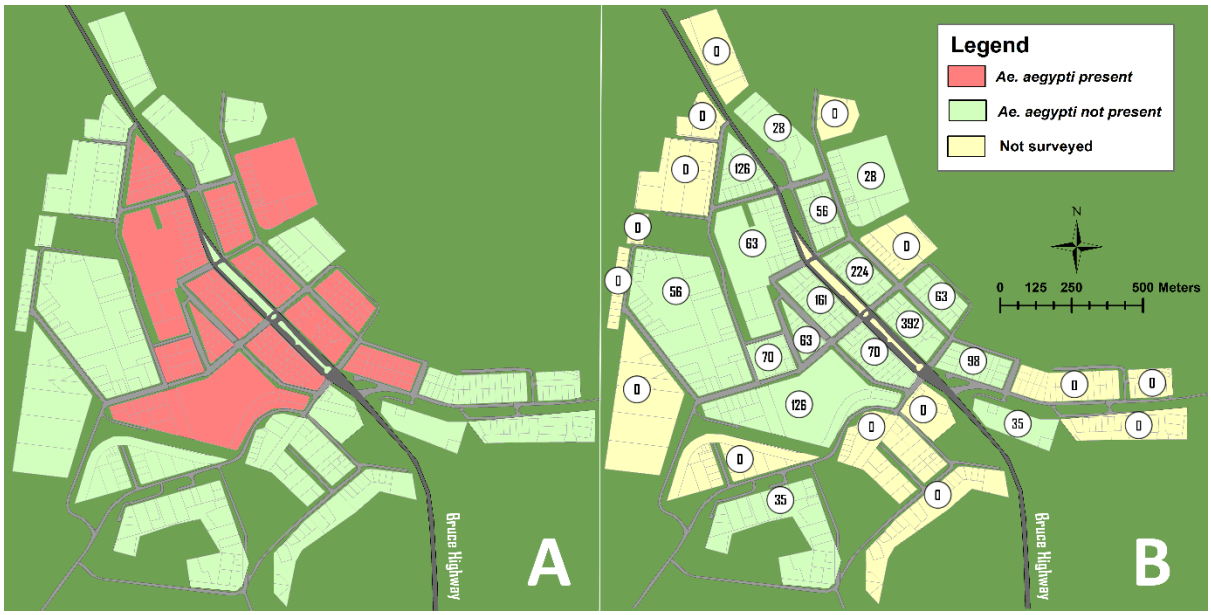

Figure 4. Blocks surveyed for *Aedes aegypti* 2011-2015 (A) and trapping 2016-2020 (B) in Gin Gin, Australia. Circled numbers represent the cumulative number of trap days across the town form 2016-2020 suggesting the species is no longer present (B).

Discussion

In Australia, the prevention of exotic vector-borne disease is a public health matter of national importance. A key component to understand disease transmission risk is access to data of the current distribution and abundance of vector species within different spatio-temporal scales, that range from local contact case addresses, larger environs of town or city and regional perspectives. Additionally, contemporaneous surveillance in regions that are vulnerable to stochastic invasion by urban vectors is required to enable the timely triggering of eradication campaigns as a strategy to avoid scenarios where cryptic outbreaks result from the belated recognition of covert incursions by vectors. However, the logistical challenges to obtain these data and perform eradication protocols is significant. In 2011, *Ae. aegypti* was re-detected in Gin Gin, a small regional town on a major highway into SEQ (a region which contains ~70% of Queensland's population). Infestations in towns on the margins of SEQ, particularly those that are also located on major transport pathways, increase the risk that *Ae. aegypti* could re-invade major population centres such as Brisbane (150 km further south) and transmit large epidemics of dengue (7). Gin Gin provides a case study of the sustained and concerted public health effort, involving both traditional mosquito control and innovative entomological surveillance during the period 2012-2020, that is required to obtain confidence that an *Ae. aegypti* population was suppressed below the level of detection in a small town. This important public health outcome demonstrates that traditional mosquito control is effective at suppressing and potentially eliminating *Ae. aegypti* populations in small towns. However, it should also serve as a warning that traditional methods may not be sustainable for larger towns and cities that will intensify both the spatial (heterogeneous distributions resulting from low dispersal behaviours) and temporal challenges (drought-resistant eggs) for surveillance sensitivity. This will be particularly difficult without investment in national capacity and capability to perform large scale interventions. The logistical resources and costs to scale our model to large urban areas (40) is significant and suggest strategic planning (20) to incursions should embed genetic analyses and additional innovative measures within

routine entomologic surveillance and emergency responses, and include *Wolbachia* or emergent technologies that are not insecticide based.

Managing the Gin Gin detection benefited from health authorities and scientists partnering to conduct regular entomological surveillance and control activities. During summer 2015/2016, the informal technical advisory group had oversight of key mosquito control activities which integrated source reduction (sealed rainwater tanks) and treatment of larval habitat with residual insecticides in the central business and residential areas of the town. This effectively suppressed the population to levels below detection thresholds by traditional surveillance methods. By late summer 2019/2020 and after four years of surveillance activities, *Ae. aegypti* was not detected in Gin Gin. The 2015/2016 intervention was the culmination of sustained and concerted effort by public health authorities and the community to destabilize the *Ae. aegypti* population. This effort included several local initiatives:

1) Effective engagement with the local community, which were highly supportive of mosquito surveillance activities, and ensured ongoing compliance with health authorities;
2) Ongoing surveillance that identified key rainwater tanks acting as major urban mosquito population sources; and
3) Consistent pressure/focus from local government that identified rainwater tanks non-compliant with mosquito regulations were drained, sealed or removed which removed egg banks and ensured larval habitat was unavailable during periods of low rainfall.

Aedes aegypti was initially re-detected in a single block in northern Gin Gin during a routine larval survey. Interestingly, two similar surveys had not previously identified the species in the town. The 2011 detection suggests the population may have persisted at very low levels or been recently re-introduced. Such uncertainty highlights the logistical challenges of traditional house-to-house, presence-absence surveillance for urban mosquitoes that can persist for extended periods as drought-resistant eggs. This species exhibits low movement over a lifetime (<200 m), however, *Ae. aegypti* and other anthropophilic species utilise human-mediated transportation thereby facilitating long-distance

dispersal (41). This long-distance dispersal may be one factor that contributed to the first detection of the species within the northern area of the town. Several reasons could be hypothesised for the 2011 re-detection of the species. The positive residential block contains the local showground which hosts a constant flow of travellers who overnight in campervans. There is also a large commercial trucking stop at the northern end of Gin Gin, two blocks from the detection where large numbers of trucks stay overnight after travelling for extended periods from areas where *Ae. aegypti* is abundant. Mosquitoes may have entered a campervan or truck freight and 'hitchhiked' from northern or central Queensland to Gin Gin. Alternatively, the detection is a remnant historical population last detected in routine house-to-house surveys in 1986. The determination of point-of-origin requires access to high resolution, genomic sequencing techniques and a separate analysis is currently being undertaken on *Ae. aegypti* populations in the region.

A key result of the genetic analysis indicated that the *Ae. aegypti* within Gin Gin formed a homogeneous cluster with a small effective population size. The resolution of the analysis did not differentiate whether this population was newly established in 2011 or a relict population. However, the result concluded that the population is vulnerable to elimination measures given the very low level of genetic admixture, low effective population size and level of inbreeding. Furthermore, the low genetic diversity and degree of differentiation observed between the Gin Gin *Ae. aegypti* population and other Queensland samples suggest a low likelihood of reinvasion from central Queensland. These findings suggest that all new incursion events (e.g. Tennant Creek invasion of 2021) should be genetically analysed to determine whether *Ae. aegypti* genetic profiles are characterised as invasive or not. Our evidence suggests that the Gin Gin *Ae. aegypti* population was vulnerable to elimination measures. Furthermore, this genetic catalogue of the *Ae. aegypti* population is important to determine the long-term effectiveness of suppression outcomes. It will provide a definitive answer to whether future detections are from the original population or introduced from separate populations that may or may not be 'invasive'. Characterization of genotypes from all Queensland population centres will inform a point-of-origin assessment, potentially from highly invasive genotypes, within

391 SEQ and other Australian incursions. Genomic catalogues will also provide a reference point for
392 potential breaches at international first points of entry.

393 Elimination campaigns that have used traditional forms of urban mosquito control typically involve
394 community education, source reduction, and residual insecticides have been effective at eliminating
395 *Ae. aegypti* populations in Australia. For example, the first elimination campaign in Australia removed
396 *Ae. aegypti* from Brisbane and surrounding areas during the mid-twentieth century (5) when the city
397 population was much smaller. The species was eliminated via effective anti-mosquito regulations
398 which targeted larval habitat such as unsealed rainwater tanks (5, 12). More recently, the species has
399 been eliminated from the small and isolated communities of Groote Eylandt (13) and Tennant Creek
400 (14, 42) in the Northern Territory. It is likely that Australia's low rainfall contributed to the long-term
401 and permanent suppression of those populations. A novel method of population suppression under
402 low rainfall conditions is the application of *Wolbachia* that exploits the deleterious effects of certain
403 strains (27, 43). This strategy utilizes the loss of desiccation resistance in *Aedes* eggs to eliminate a
404 population over extended dry periods. This 'replace and suppress' strategy would not only prevent
405 dengue transmission, but is likely to be highly effective for suppressing, populations in large urban
406 settings that will otherwise prove difficult logistically to inspect and treat with insecticides,
407 exacerbated by non-treatment of cryptic larval habitat (44). Utilizing the wet-dry seasonal dynamic of
408 Australian landscapes will be important to future campaigns which seek to eliminate populations of
409 container inhabiting vectors like *Ae. aegypti* and *Ae. albopictus*.

410 In Queensland, the prevention of dengue and controlling vector species is the shared responsibility of
411 both state and local government organisations. Queensland Health has the overall responsibility
412 under the *Public Health Act 2005* for the control of communicable diseases in Queensland, including
413 exotic mosquito-borne diseases such as dengue fever. Provisions within Chapter 2 of the *Public Health*
414 *Act 2005* provide local governments with the statutory support and powers to undertake mosquito
415 surveillance and control activities (via insecticide treatment) and to prevent and control public health

risks in relation to mosquitoes within residents' premises. This involves the Queensland Health chief executive sanctioning an authorised prevention and control program when an area is likely to contain an infestation of a disease vector such as *Ae. aegypti* or risk of an outbreak of vector-borne disease. For example, unmaintained rainwater tanks can be made to comply with the *Public Health Regulation 2005* and *Public Health Act 2005* by local authorities, so they no longer function as larval habitat for *Ae. aegypti* or other mosquito species. These essential powers were drawn upon during the period 2011-2020 to ensure residents in Gin Gin did not continue to store or removed containers where *Ae. aegypti* was present. Importantly however, the logistical challenge for health authorities to access and eliminate mosquitoes in all homes and businesses during control activities in very large towns and cities using chemical models of elimination is immense. Thus, a central element of urban mosquito control is to raise awareness about the community's role to adopt behaviours that eliminate mosquito breeding at home and in the workplace. Concurrent investment is required to provide baseline monitoring programs that are more representative of the spatio-temporal parameters of urban mosquitoes to establish entomological confidence in a negative result for invasive species. Current use of surveillance programs that increase throughput and sensitivity by the use of molecular diagnostic platforms (RSVP) and that can be linked to citizen science platforms (Mozzie Monitors (45) and Zika Mozzie Seeker (46)) can provide opportunities to further increase sampling frequency and site number will inform a detection threshold.

Establishing and maintaining community support in Gin Gin was essential to the success of urban mosquito control and dengue prevention initiatives. An effective engagement strategy sets objectives and defines the underlying activities that will best meet these objectives and those of the project. Utilizing council and health officers from the local community promoted and encouraged local acceptance, ownership of the project's goals, and facilitated entomologic surveillance and control activities. Trust is an important element of community engagement which must be established and maintained to ensure community support throughout the life of the intervention. The formation of a community reference group during 2015-2016, was essential to building trust during the intervention

when rainwater tanks were sealed, and insecticides utilized for suppression. A community reference group can provide a social licence to operate and facilitates the transfer of information from scientists or health authorities to the community or opportunities for community concerns to be voiced. Efforts to ensure a comprehensive engagement strategy can foster increased community acceptance, provide local support for activities and even some level of ownership as it promotes both enthusiasm within the community and adherence to personal behaviours. Effective acts that reduce urban mosquito breeding sites in residential premises and significantly reduce the vulnerability of individuals and community to invasive urban species and associated diseases.

Conclusion

The extensive public health efforts documented here demonstrate that an integration of traditional mosquito control, a small genetically isolated mosquito population and public engagement can eliminate *Ae. aegypti* from a small regional town. Replicating this model for large towns and cities at appropriate spatial-temporal scales that will provide an early warning capability and monitor the efficacy and longevity of suppression activities may prove extremely difficult to sustain without incorporating innovative solutions. Removing vectors from a region is a strategic solution to preventing disease transmission. Re-emergence of dengue in Rockhampton after 60 years is a reminder that wild-type populations of *Ae. aegypti* in Queensland still represent a risk of disease transmission. While SEQ region is currently considered vector-free, this Gin Gin case study demonstrates that there is cause for caution. Detection thresholds are insensitive throughout much of the region and stochastic incursions risks remain via freight connections with established *Ae. aegypti* Queensland populations, and increased interceptions at international First Ports of Entry (airports and seaports). This regional risk is heightened by over 300,000 rainwater tanks installed throughout SEQ which are generally unmonitored by authorities and are approaching the end of their warranty periods. Engaging communities to participate in surveillance (citizen science) may also encourage broader awareness and adoption of personal behaviours that reduce availability of

residential and commercial sites to urban mosquito species that will also reduce regional vulnerability to invasive species and associated risk of exotic diseases, particularly in regions with a high number of viraemic travellers and/or proximal to ports of entry. Application of the Gin Gin model to large towns and urban cities of SEQ and Australia will require significant investment in national capacity and capability. Robust and contemporaneous urban mosquito surveillance programs are required that are expansive and sustainable to provide the level of sensitivity required to provide regional confidence that towns and cities are absent of vectors and sensitive enough to detect incursions (that may be focal for many years) relatively quickly. This capability is enhanced by linking surveillance methods to molecular diagnostic methods to develop genetic reference libraries to define species identification, point-of-origin and insecticide resistance. In turn, this investment will build essential experience and baseline monitoring data that will inform elimination strategies if invasive vectors such as *Ae. aegypti* or *Ae. albopictus* are detected in major Australian cities.

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Supporting Information

S1 Appendix. Queensland Health Report: Population suppression of Aedes aegypti in Gin Gin, Bundaberg, Queensland. (re distributed through a Creative Commons Attribution 3.0 Australia licence).

S2 Appendix. Community Engagement Plan

S3 Appendix. Risk Management Strategy

S4 Appendix. Mosquito Suppression Plan

S5 Appendix. T1. Samples of *Aedes aegypti* larvae. T2. Number of genetic clusters assigned by STRUCTURE of *Aedes aegypti* from Gin Gin, Queensland. F1. Genetic clusters assigned by STRUCTURE for samples of *Aedes aegypti* from Queensland, Australia.

S6 Appendix. *Aedes aegypti* positive residential blocks in Gin Gin, Queensland F1. 2012-2013, F2. 2013-2014, F3. 2014-2015, F4. 2015-2016, F5. 2017-2018, F6. 2018-2019, F7. 2019-2020.

Data reporting

The datasets supporting the conclusions of this article are included within the article (and its additional files).

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Competing interests

There are no competing interests, whether they be financial, personal or professional, that have influenced the work.

Human and animal research

Human ethics approval for the 2015-2016 community engagement, mosquito suppression and research was provided by the CSIRO Health and Medical Research Human Research Ethics Committee (Proposal #12/2015) and QIMR Berghofer Human Ethics Committee (#P2054).

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