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Egg density as a guide for *Aedes* control: a controlled field comparison of four intervention strategies in a Brazilian city

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Abstract

Brazilian municipalities control *Aedes aegypti* with a routine of bimonthly visits to every premise, and when counts stay high despite that routine, agents have little evidence on what to add. We compared four additional actions, each directed by ovitrap counts, in Patos de Minas, Minas Gerais. Five of the city's eleven operational zones were followed fortnightly for two years, all on the routine programme throughout. In the second year four zones also received one additional action within 150 m of traps holding more than 50 eggs: extra visits for container elimination with focal *Bacillus thuringiensis israelensis* (Bti), the same plus ultra-low-volume (ULV) spraying, clean-up campaigns, or ULV alone. The fifth kept the routine only. We examined 4,670 ovitraps and counted 165,199 *Aedes* spp. eggs. In a difference-in-differences negative binomial model with autocorrelation-consistent errors, no action differed significantly from the routine alone. The largest reduction followed extra elimination with Bti (rate ratio 0.53, 95% CI 0.25–1.13) and partly predated the change; ULV alone had the least favourable estimate (1.12, 0.49–2.53). Rainfall accounted for more deviance than all four actions together. The population was resistant to all four pyrethroids

tested. With one zone per action these results are exploratory, but they point to larval work rather than spraying as the addition to test first.

Keywords: *Aedes aegypti*, ovitrap, larval source reduction, *Bacillus thuringiensis israelensis*, ultra-low-volume spraying, insecticide resistance

Key message

- Ovitrap counts directed and evaluated four actions added to the routine dengue programme.
- Extra container elimination with Bti gave the largest drop in egg density, short of significance.
- ULV spraying, alone or added to larval work, showed no detectable benefit.
- Rainfall accounted for more variation in egg density than the four actions combined.
- The local *Ae. aegypti* population was resistant to all four pyrethroids tested.

1 Introduction

Aedes aegypti (Linnaeus, 1762) transmits dengue, chikungunya, Zika and urban yellow fever, and its range keeps widening (Kraemer et al. 2015; Lim et al. 2025). The species breeds in small artificial containers around dwellings and bites by day, traits that make it an efficient vector and a stubborn target (Consoli and Louren¸co-de Oliveira 1994). Brazil registered 6,563,561 probable dengue cases and 6,321 deaths in 2024, the heaviest burden ever recorded in the country. Minas Gerais alone accounted for 1,658,506 of those cases, a 154% increase over 2023. The recent chikungunya resurgence across South America has exposed how little entomological information most municipal programmes can act on (Corbel et al. 2026). Beyond the clinical toll, dengue cost Brazil an estimated US$1.35 billion a year in direct and indirect costs over 2000–2007 (Teich et al. 2017). Vaccines exist but reach narrow age bands, so control still rests on the vector (Kalimuddin et al. 2025).

The Brazilian National Dengue Control Programme prescribes bimonthly house-to-house visits, elimination or larvicidal treatment of containers, and space spraying of insecticide around notified cases (Brasil, Minist´erio da Sa´ude 2009). Municipalities execute this menu with little local evidence about which component makes a difference. Reviews of space spraying have repeatedly found weak or absent effects on vector populations (Esu et al. 2010; Bonds 2012), yet ultra-low-volume (ULV) application remains the most visible response to an outbreak. Bti larviciding has better support (Boyce et al. 2013), and the integrated programmes that did reduce vector popula-tions in Brazilian cities combined several actions rather than relying on one (Regis et al. 2013; Macˆedo et al. 2021). Insecticide resistance complicates the picture further. Brazilian *Ae. aegypti* populations have been resistant to pyrethroids for two decades (Braga and Valle 2007; Valle et al. 2019; Melo Costa et al. 2020), which raises an obvious question about spraying a pyrethroid-containing product at all.

Evaluating any of this requires a measurement that responds fast enough to matter. The larval indices used in routine surveillance are qualitative, expensive in agent-hours, and poorly correlated with adult abundance or transmission risk (Bowman et al. 2014). Ovitraps offer an alternative. A dark water-filled container with a rough paddle attracts gravid females (Fay and Eliason 1966), and they draw ovipositing females better than larval traps even where natural breeding sites abound (Silva et al. 2009). Egg counts track vector population fluctuations more sensitively than any other trap compared side by side in Brazil (Codec¸o et al. 2015). Egg density has predicted dengue outbreaks two months ahead in Belo Horizonte (Pessanha et al. 2014) and maps onto the local distribution of cases (de Albuquerque et al. 2018). A systematic review of Brazilian ovitrap studies nonetheless found protocols varying widely between municipalities, which limits comparison across sites (Fernandes Silva Chagas do Nascimento et al. 2025). Brazil's Ministry of Health formalised ovitrap surveillance in 2022 (Brasil, Minist´erio da Saúde 2022), and municipalities now record counts in a national platform that produces egg-density heat maps without local GIS expertise (Conta Ovos 2026).

That combination makes a different experiment possible. Agents who already visit every premise every two months, as the national programme requires, still face blocks where density does not fall, and have little local evidence on what to add. If egg density is sensitive enough to detect a change in the vector population, it can decide where an additional action should go and then judge whether it worked. In Patos de Minas, Minas Gerais, we kept the routine programme running in five zones for two years and, in the second year, added a different ovitrap-guided action in four of them. With one zone per action the study is exploratory. We ask which additional action looks most worth testing in a replicated trial.

2 Materials and methods

2.1 Study site

Patos de Minas lies in north-western Minas Gerais, Brazil. The municipality has 159,235 inhabitants over 3,190.5 km^2 (Instituto Brasileiro de Geografia e Estat´ıstica 2023). The predominant biome is the cerrado, the climate is tropical highland, mean temperature is 21 °C, and most rain falls between October and April. Basic sanitation coverage is high: 92.6% of the population receives piped water, 93.3% is connected to a sewerage network, and 96.1% has refuse collected.

Dengue is recurrent here. Epidemics occurred in 2016, 2019 and 2022 on a roughly triennial pattern, then in three consecutive years. In 2023 the municipality notified 12,584 cases and confirmed 7,469. In 2024 it notified 21,209, confirmed 13,524, and recorded six deaths. The January 2024 rapid larval survey returned a house index of 5.9, which classifies the municipality as high risk.

Following the geographical reconnaissance required before any control activity (Brasil, Minist´erio da Saúde 2009), the urban perimeter is divided into 11 operational zones covering 98,325 premises (Fig. 1).

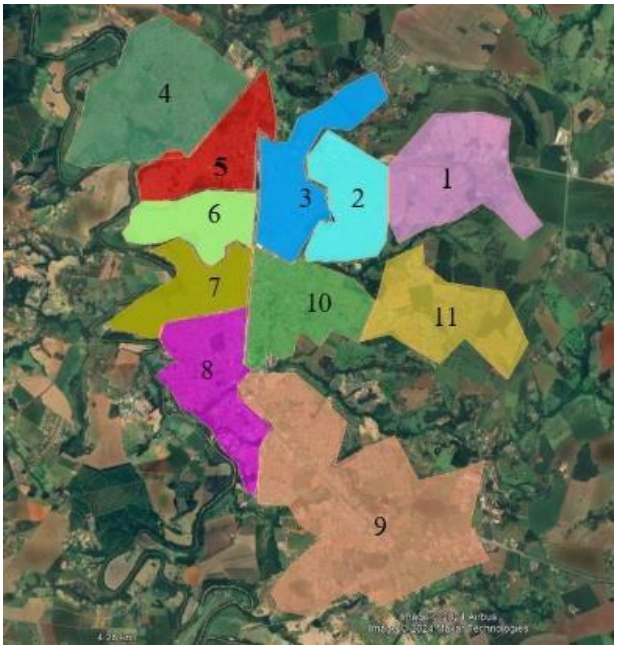


Fig. 1 The 11 operational zones of the urban perimeter of Patos de Minas, Minas Gerais, Brazil, delimited by geographical reconnaissance. Five were followed in this study. All five kept the routine programme throughout. In the second year zone 2 added source reduction with Bti plus ULV, zone 5 source reduction with Bti, zone 8 clean-up campaigns and zone 11 ULV alone, while zone 6 served as control

2.2 Study zones and design

We selected five of the 11 zones (Table 1). Zones were chosen far enough apart that an action in one would not spill into another; zones 5 and 6 adjoin on the map but are separated by a watercourse. Strategies were assigned to zones without a random draw and without any criterion linking a zone to its strategy.

The design is a controlled before-and-after comparison. Throughout both periods all five zones ran the routine national programme. Agents visited every registered premise once every two months, eliminated containers and treated those that could not be removed, and space spraying followed notified dengue cases. In the first period, from epidemiological week (EW) 11 of 2023 to EW 12 of 2024, nothing was added. In the second, from EW 14 of 2024 to EW 12 of 2025, zones 2, 5, 8 and 11 each received one additional action directed by egg counts (Section 2.4), and zone 6 kept the routine alone as control. The additional actions are increments on top of the routine, not replacements for it. Educational sessions continued city-wide in both periods. Zone characteristics were taken from the national programme database (SisPNCD) and resident counts from the primary-care register of the municipal health department.

SisPNCD production reports for April 2024 to March 2025 show how much house-visit work each zone actually got. Visits ran close to the bimonthly target everywhere, at 5.1 (zone 11), 5.4 (zone 2), 6.4 (zone 5), 6.7 (zone 8) and 7.2 (zone 6) visits per

Table 1 The five study zones: additional action, premises registered in 2024 and resident population

Zone	Additional action	Residences	Commercial	Vacant lots	Strategic points	Total premises	Residents
2	Source reduction + Bti + ULV	5,635	1,026	655	0	7,950	13,537
5	Source reduction + Bti	6,481	775	749	6	8,842	15,127
6	None (control)	4,676	2,408	605	3	8,689	13,885
8	Clean-up campaigns	5,950	1,127	475	33	8,982	14,372
11	ULV spraying alone	5,178	445	1,776	0	7,989	12,224

Additional actions ran within 150 m of traps holding more than 50 eggs, on top of the routine programme, which continued in all five zones. Premises counts come from the national programme database (SisPNCD) and exclude the "other" category, so the columns shown do not sum to the total. Strategic points are premises such as scrapyards and recycling depots that hold many containers. Zone 6 has the largest share of vertical buildings, whose upper floors are not counted in geographical reconnaissance, so its residence count understates housing units.

registered premise per year. In zones 2 and 5 these figures include the additional ovitrap-triggered visits, which agents entered in SisPNCD under the same activity as routine ones. Even so, the control zone was visited most. Refusals were under 1% of visits in every zone, but premises found closed ranged from 11% of visits in zone 8 to 24% in zone 5. Educational sessions took place in schools and in primary health unit waiting rooms, where agents showed larvae, adults and eggs on paddles and explained how to find and remove containers.

2.3 Ovitrap surveillance

Ovitraps have been deployed across the whole urban perimeter since 2018. Each trap is an 800 mL black plastic cup holding 300 mL of tap water and 1 mL of a 12% brewer's yeast suspension, giving a final concentration of 0.04% (Lima 2022; Brasil, Minist´erio da Saúde 2022). A 12.0 × 3.0 cm Eucatex plywood paddle is clipped inside with the rough face inwards. Paddles were soaked for 24 h to remove sawdust, dried for a further 24 h, then numbered and dated before installation.

Agents installed traps in the peridomestic area at up to 80 cm above ground, shaded, sheltered from rain, and out of reach of children and animals. Spacing followed the national technical note at 200 m between traps (Brasil, Minist´erio da Saúde 2022). Traps stayed in the field for seven days and were set fortnightly, giving two obser-vations per month. Recovered paddles were dried for at least 24 h and eggs counted under a stereomicroscope. Eggs were counted as *Aedes* spp.; they were not hatched or identified to species, so the counts may include *Ae. albopictus*. Counts were entered into SisPNCD and into the Conta Ovos platform (Conta Ovos 2026), which georefer-ences each trap, computes the indices below, and renders egg-density heat maps with a kernel radius of approximately 574 m.

Three indices were computed per zone and round. The mean ovitrap index (MOI) is eggs divided by ovitraps examined. The egg density index (EDI) is eggs divided by positive ovitraps. The ovitrap positivity index (OPI) is the percentage of examined ovitraps containing at least one egg. MOI is the outcome used throughout; it is the

only one of the three that is a density over the full sample, so it responds to both a fall in the number of ovipositing females and a fall in eggs laid per trap.

2.4 Interventions

Heat maps generated by Conta Ovos identified the traps with the highest egg counts each round. Any trap holding more than 50 eggs triggered the zone's additional action within roughly 150 m of it, about nine city blocks. Actions ran between EW 14 of 2024 and EW 13 of 2025, 13 rounds in all, roughly one every four weeks and in the same weeks in all four intervention zones. The four additional actions were:

- **Zone 2, source reduction with Bti plus ULV.** On top of their routine visits, agents revisited premises within the radius, eliminated containers, and treated those that could not be removed with VectoBac® WG (*Bacillus thuringiensis israelensis* strain AM65-52, 37.4%) at one 1 g dosing spoon per 100 L of water. Five ULV cycles at three-day intervals were applied on top of this.
- **Zone 5, source reduction with Bti.** The same additional visits, container elimination and focal Bti treatment, without spraying.
- **Zone 8, clean-up campaigns.** Agents ran 13 collective clean-up drives and removed discarded material from the radius, mostly household refuse and rubble, with some tyres. The volume was small enough to go out with the regular municipal refuse collection; no dedicated trucks were needed.
- **Zone 11, ULV spraying alone.** Five ULV cycles at three-day intervals within the radius, with no additional larval work.

In zones 2 and 5, the containers removed were mostly movable ones (plant-pot saucers, buckets, cans) and tyres. Those treated with Bti were mostly ground-level water stores such as drums, and fixed containers built into the premises.

SisPNCD does not record containers by the action that dealt with them, but it does record, for every zone, the premises given focal larvicide treatment. The ratio of those premises to visits made tracks the larval work. Over April 2024 to March 2025 they were 14.1% of visits in zone 5, 9.4% in zone 8, 8.5% in zone 6, 6.5% in zone 2 and 2.7% in zone 11. Comparing the same months a year apart removes the season. From January to March 2024 to January to March 2025, premises treated rose by 94% in zone 2 (516 to 1,000) and 31% in zone 5 (1,614 to 2,112), against 22% in the control zone. They rose 15% in zone 11 and fell 9% in zone 8, neither of which had additional larval work.

Space spraying used Cielo® (imidacloprid 30 g kg^{-1} and prallethrin 7.5 g kg^{-1}) at 100 mL ha^{-1}, the adulticide then in use by the national programme. Two Guarany Leco 1800 E vehicle-mounted machines were calibrated in June 2024 to a flow of 75 mL min^{-1} and driven at 15 km h^{-1}. Flow was checked by running each machine into a graduated cylinder for one minute. Droplet size was measured with a KDL DC-IV analyser. Volume median diameters were 20.52 and 19.26 μm, within the range specified by the Ministry of Health (Brasil, Ministério da Saúde 2020). City-wide ULV in response to notified dengue cases continued in all five zones throughout.

2.5 Insecticide susceptibility

Positive paddles from EW 18 of 2024, drawn from all five study zones and pooled, were sent to LBCVIV/Fiocruz in Rio de Janeiro and reared to the F2 generation. Diagnostic-concentration bioassays used WHO impregnated-paper tubes for deltamethrin (0.03%), permethrin (0.4%) and malathion (1.5%), and impregnated bottles for prallethrin (30 µg per bottle), transfluthrin, imidacloprid and flupyradifurone (World Health Organization 2016, 2022). Each assay used four exposure tubes or bottles and two controls, with 20 to 25 three-day-old females each, giving 80 to 100 females per assay. At least three assays were run on separate days per compound. Knockdown was scored one hour after exposure and mortality at 24 h, in a room held at 26 ± 2 °C and 70 ± 15% relative humidity. Populations were classified as susceptible above 98% mortality, possibly resistant between 90 and 98%, and resistant below 90%. The Bti formulation used in zones 2 and 5 was not tested.

2.6 Meteorological data

Daily minimum temperature and rainfall came from the national meteorological institute station for the municipality (Instituto Nacional de Meteorologia 2025). Values were averaged over each fortnightly trap interval to match the entomological series, then standardised as z-scores.

2.7 Statistical analysis

Analyses were run in Python 3.12 with statsmodels 0.14 (Seabold and Perktold 2010). Two questions were kept separate: whether the zones differed at baseline, and whether the actions changed anything.

For the first, one-way ANOVA compared mean MOI across the five zones within each period, with Tukey HSD for pairwise contrasts at $\alpha = 0.05$.

For the second, we fitted a difference-in-differences model (Wing et al. 2018). Egg counts per zone and round were modelled with negative binomial regression (Hilbe 2011), with log(ovitraps examined) as offset so that the response is egg density rather than a raw count, and with zone, period and their interaction as predictors. Zone 6 is the reference level and the first period is the reference period, so each zone × period interaction term estimates the change in that zone net of the change seen in the control zone over the same weeks. Standardised minimum temperature and rainfall were added as covariates. The dispersion parameter was estimated from the Pearson residuals of a Poisson fit. Coefficients are reported as rate ratios with 95% confidence intervals.

The same zone is observed every fortnight, so residuals are correlated in time, and errors that ignore this overstate precision in difference-in-differences designs (Bertrand et al. 2004). Standard errors are therefore Newey–West heteroskedasticity- and autocorrelation-consistent estimates computed within zone, with two lags, about one month (Newey and West 1987). Four actions were tested against one comparison zone, and we report Holm-adjusted p-values alongside the unadjusted ones (Holm 1979). The four comparisons follow directly from the design, but no analysis plan was registered.

We checked the model in five ways (Table 4). Zone-specific linear trends fitted to the first period test whether the zones were already diverging from zone 6 before the change. An event study splits each period into halves. A placebo analysis moves the start date to the middle of the first period, where no action was running. We refitted with four and six lags, with heteroskedasticity-robust (HC1) errors only, and with the previous round's egg density as a covariate. Finally, we compared actions directly by contrasting their interaction terms, which is the comparison that ranks them. To put covariates and actions on a common scale, we report the increase in deviance when each is dropped from the model. With five zones, none of these checks can remove zone-level confounding.

We depart here from the interrupted time-series approach used in the source dissertation (Bernal et al. 2017; Hyndman and Athanasopoulos 2021). Fitting an ARIMA(0,1,0) model with a step dummy for the intervention returned standard errors of zero and p-values below 0.001 for every zone, including the control zone, which is a numerical artefact of differencing a series against a step change rather than a find-ing. The difference-in-differences specification answers the same question and uses the control zone rather than a model extrapolation as the counterfactual.

2.8 Ethics

The study analysed secondary entomological data with no personal information and used the methods already applied by the municipal *Ae. aegypti* control programme, so it did not require review by a human research ethics committee. Residents of premises where traps were installed signed an informed consent form.

3 Results

3.1 Sampling

Across 49 fortnightly rounds we examined 4,670 ovitraps in the five zones and counted 165,199 *Aedes* eggs. Table 2 gives the deployment per zone. Losses were low. During the intervention period, 68 of 2,574 scheduled traps (2.6%) were not recovered, because residents were absent at collection, or the trap or paddle had been removed, broken, dried out or flooded. Zone 2 accounted for 27 of those 68, and there losses were not independent of density: during the intervention period, rounds with fewer traps recovered had higher MOI (Spearman $\rho = -0.40$, $p = 0.041$). The offset corrects for how many traps were read, not for which were lost.

3.2 Baseline differences between zones

The five zones did not start from the same place. Under the routine programme alone, mean MOI differed significantly between them (ANOVA, $F = 10.31$, p ¡ 0.001). Tukey HSD separated a low-density pair from a high-density trio: zones 2 and 11 averaged around 20 eggs per trap examined, while zones 5, 6 and 8 averaged close to 50. Every contrast between a member of the low pair and a member of the high trio was significant ($p \leq 0.005$), and no contrast within either group was.

Table 2 Ovitrap deployment and losses per zone

Zone	Traps per round	Rounds	Traps examined, period 1	Traps examined, period 2	Not recovered, period 2
2	17	49	362	415	27 (6.1%)
5	19	49	497	490	4 (0.8%)
6	19	49	430	471	23 (4.7%)
8	24	49	422	615	9 (1.4%)
11	20	49	453	515	5 (1.0%)
Total	99	49	2,164	2,506	68 (2.6%)

Period 1 is EW 11/2023 to EW 12/2024 (23 rounds), period 2 is EW 14/2024 to EW 12/2025 (26 rounds). “Traps per round” is the number scheduled; “traps examined” counts paddles actually recovered and read.

This matters for interpretation. Zones 2 and 11 had little egg density to lose before their interventions began, and they are precisely the two zones assigned to receive ULV.

During the intervention period the zones still differed ($F = 5.08$, $p = 0.0008$), but less sharply, and only three contrasts survived Tukey correction (zone 2 versus zone 6, mean difference 27.9, $p = 0.003$; zone 2 versus zone 8, 23.5, $p = 0.021$; zone 6 versus zone 11, 24.2, $p = 0.016$).

3.3 Egg density before and after

Figure 2 shows MOI in each zone across all 49 rounds. The seasonal signal dominates every panel: density collapses through the dry southern winter and rebuilds from around EW 44 as the rains return, peaking in January and February. Any intervention effect has to be read against that.

Zone 5 fell furthest. Its mean MOI dropped from 58.1 to 29.4, its positivity from 62.1% to 44.9%, and its egg density index from 85.6 to 54.4 (Fig. 3). The control zone moved little, from 48.3 to 45.7, and its positivity rose from 56.8% to 62.1%. Zone 8 fell from 50.6 to 41.3. Zones 2 and 11 had low densities in both periods and changed little in either direction, zone 2 from 19.7 to 17.8 and zone 11 from 20.1 to 21.4.

3.4 Effect of the interventions

Comparing each zone against the control zone over the same weeks separates the actions from the season. Table 3 and Fig. 4 give the estimates.

Zone 5, with additional source reduction and Bti, showed the largest relative reduction. Its egg density fell to 0.53 of what the control zone’s trajectory implies, a 47% drop. The interval is wide and includes no effect (95% CI 0.25–1.13, $p = 0.098$; Holm-adjusted $p = 0.39$). The other three actions sat closer to one: clean-up campaigns 0.87 (0.43–1.79), source reduction with Bti plus ULV 0.92 (0.43–1.98), and ULV alone 1.12 (0.49–2.53). None differed significantly from the routine alone. Nor did any pair of actions when compared directly, so the data rank the actions without confirming the

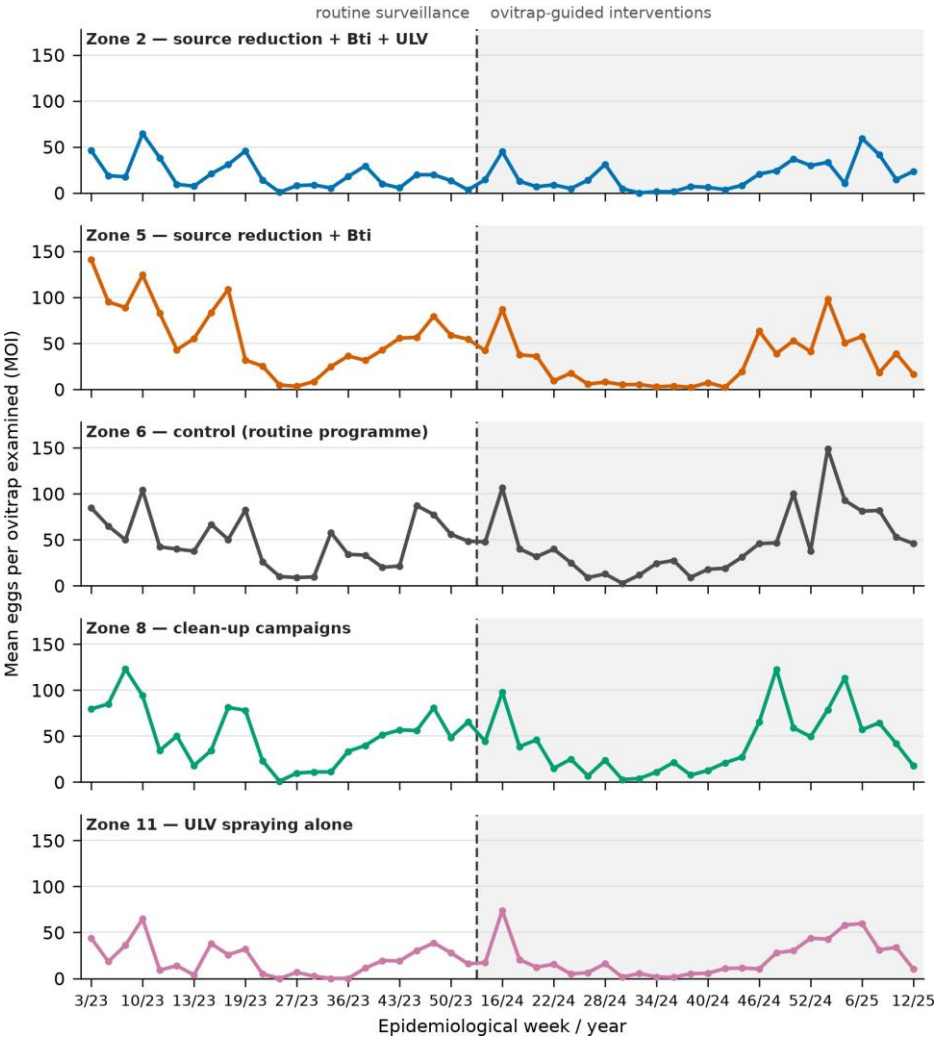


Fig. 2 Mean ovitrap index (eggs per ovitrap examined) in the five study zones over 49 fortnightly rounds. The dashed line marks the start of the ovitrap-guided interventions in EW 14 of 2024; the shaded region is the intervention period. Zone 6 received no additional action at any point; the routine programme continued in all five zones

ranking. Zone 2 against zone 5, the contrast that isolates adding ULV to the same larval work, gave 1.75 (0.77–3.97, $p = 0.18$); zone 8 against zone 5 gave 1.66 (0.76–3.64, $p = 0.20$), and zone 11 against zone 5 gave 2.12 (0.89–5.09, $p = 0.092$).

Rainfall was associated with egg density (RR 1.20 per SD, 1.10–1.30, p ¡ 0.001), minimum temperature less clearly (RR 1.13, 0.99–1.28, p = 0.065). Dropping rainfall from the model raised the deviance by 10.4 on one degree of freedom. Dropping all four action terms together raised it by 5.7 on four.

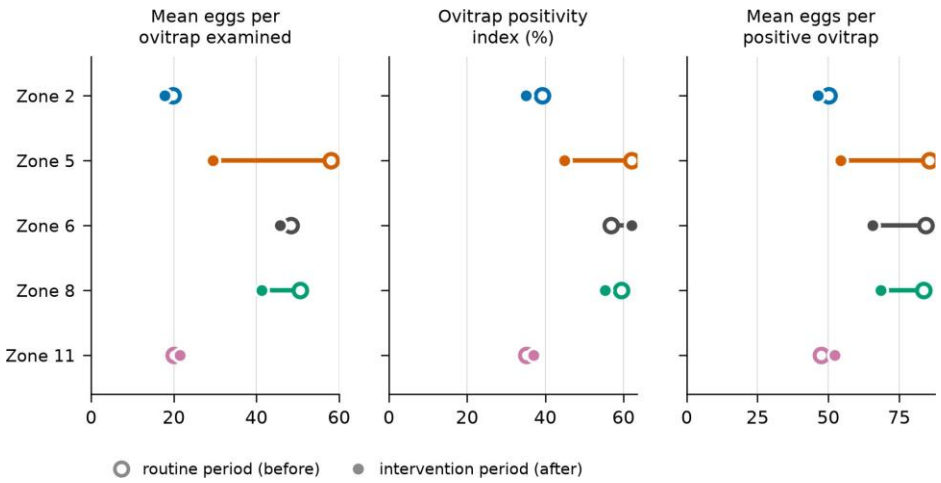


Fig. 3 Period means of the three ovitrap indices per zone. Open circles are the routine period (EW 11/2023 to EW 12/2024), filled circles the intervention period (EW 14/2024 to EW 12/2025)

Table 3 Difference-in-differences negative binomial model of egg counts, with log(ovitraps examined) as offset and zone 6 as reference

Zone	Additional action	Rate ratio	95% CI	*p*	Holm *p*
5	Source reduction + Bti	0.53	0.25–1.13	0.098	0.39
8	Clean-up campaigns	0.87	0.43–1.79	0.713	1.00
2	Source reduction + Bti + ULV	0.92	0.43–1.98	0.830	1.00
11	ULV spraying alone	1.12	0.49–2.53	0.790	1.00
Rainfall (per SD)		1.20	1.10–1.30	<0.001	
Minimum temperature (per SD)		1.13	0.99–1.28	0.065	

Each rate ratio is the zone × period interaction term, that is, the change in egg density in that zone relative to the change observed in the control zone, which kept the routine programme alone, over the same weeks. Standard errors are Newey–West heteroskedasticity- and autocorrelation-consistent, within zone, two lags; n = 245 zone-rounds. Holm p adjusts the four action terms for multiplicity.

3.5 Checks on the model

The first period gives no clear sign that the zones were diverging before the change (Table 4). Zone-specific linear trends against zone 6 were jointly non-significant (p = 0.43), although zone 2 drifted downward. Zone 5 needs a closer look. In the event study it stood at 1.33 times the control zone in the first half of period 1, 1.00 in the second half (the reference), and 0.67 and 0.58 in the two halves of period 2. A placebo start date halfway through period 1 gave it 0.75 (0.43–1.32). Part of zone 5's decline was under way before targeting began. Adding the previous round's egg density as a covariate moved zone 5 to 0.81, far from significance. Only heteroskedasticity-robust

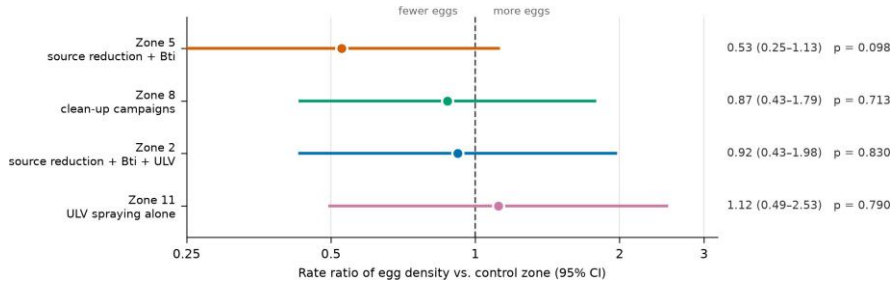


Fig. 4 Change in egg density in each intervention zone relative to the control zone, from the difference-in-differences negative binomial model in Table 3. Values below one indicate fewer eggs than the control zone's trajectory over the same weeks. Intervals use Newey–West errors within zone

Table 4 Sensitivity of the difference-in-differences estimates. Rate ratios (95% CI) for each action against zone 6

Specification	Zone 5	Zone 8	Zone 2	Zone 11
Primary: Newey–West, 2 lags	0.53 (0.25–1.13)	0.87 (0.43–1.79)	0.92 (0.43–1.98)	1.12 (0.49–2.53)
Newey–West, 4 lags	0.53 (0.22–1.24)	0.87 (0.40–1.92)	0.92 (0.40–2.09)	1.12 (0.45–2.74)
Newey–West, 6 lags	0.53 (0.21–1.30)	0.87 (0.38–1.99)	0.92 (0.38–2.20)	1.12 (0.44–2.84)
HC1 errors only	0.53 (0.30–0.93)	0.87 (0.51–1.51)	0.92 (0.49–1.73)	1.12 (0.60–2.08)
Lagged outcome (HC1)	0.81 (0.49–1.32)	0.92 (0.57–1.50)	1.02 (0.57–1.81)	0.91 (0.50–1.66)
Placebo start, period 1	0.75 (0.43–1.32)	0.98 (0.53–1.83)	0.74 (0.37–1.50)	0.91 (0.40–2.06)
Period 1 trend, per round	0.98 (0.94–1.02)	1.00 (0.96–1.04)	0.96 (0.91–1.01)	0.99 (0.95–1.04)

All models include zone, period and standardised minimum temperature and rainfall, with log(ovitraps examined) as offset. The placebo uses period 1 only, with a fictitious start at round 12 of 23. The trend row gives zone-specific linear trends against zone 6 within period 1 (joint Wald $p = 0.43$). Placebo and trend rows use Newey–West errors with two lags. Code: did sensitivity.py in the supplementary material.

errors that ignore autocorrelation made it significant (0.30–0.93, $p = 0.028$). The other three actions stayed close to one under every specification.

3.6 Spatial pattern

Heat maps from the Conta Ovos platform show where the density sat within each zone (Fig. 5). On visual inspection, the same locations in zone 5 returned as hotspots round after round, which is consistent with perennial larval habitat rather than transient containers. We did not test that persistence formally. Density did not drop in the fortnight immediately following an action; the decline appeared over successive rounds. Zone 8 tells a different story: it holds 33 of the municipality's strategic points, mostly scrapyards and recycling depots, and its northern sector stayed red for the whole intervention period despite the clean-up drives.

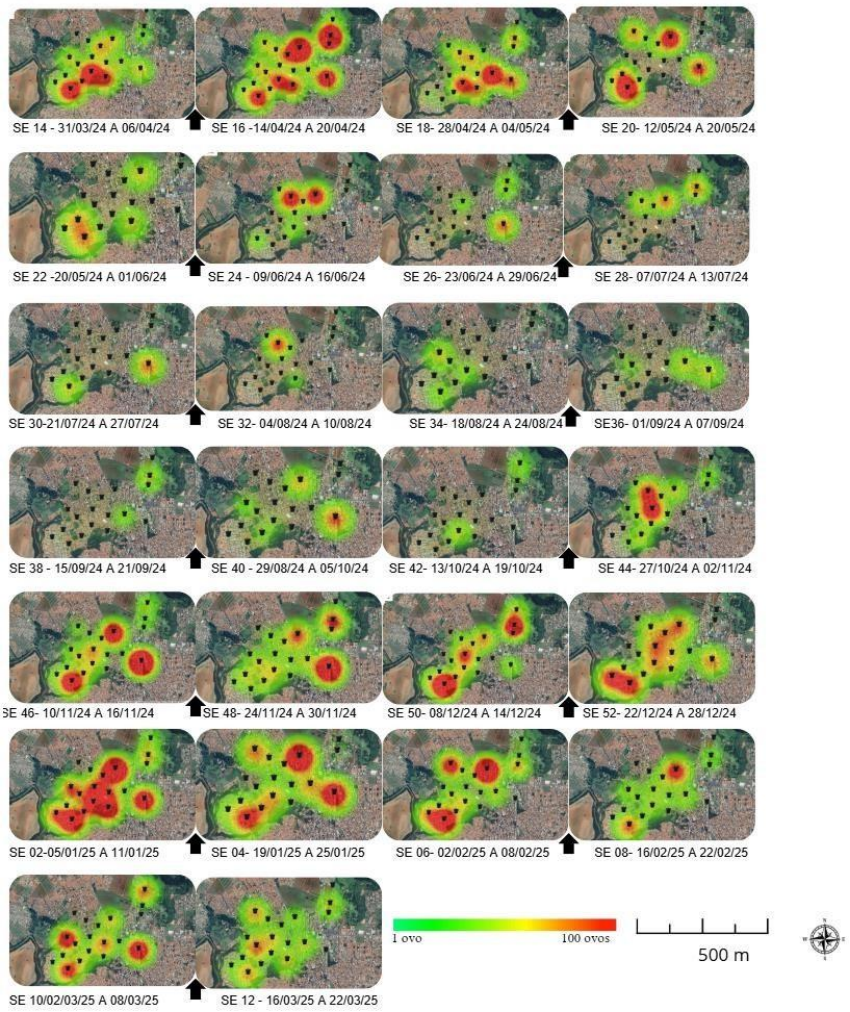


Fig. 5 Fortnightly egg-density heat maps for zone 5, generated by the Conta Ovos platform. Panels run from EW 14 of 2024 to EW 12 of 2025; arrows mark the weeks in which control actions were carried out. Green to red spans 1 to more than 100 eggs per trap. Panel labels are in Portuguese in the platform output: SE denotes epidemiological week, “1 ovo” one egg and “100 ovos” 100 eggs

3.7 Insecticide susceptibility

The local population was resistant to every pyrethroid tested: deltamethrin, permethrin, prallethrin and transfluthrin all gave mortality below 90% (Table 5). Malathion fell in the possible-resistance band. Imidacloprid and flupyradifurone were fully effective.

Prallethrin is one of the two active ingredients in the product used for space spraying in this study, and the population was resistant to it.

Table 5 Susceptibility of the Patos de Minas *Ae. aegypti* population (F2) at diagnostic concentration

Insecticide	Class	Assay	Classification
Deltamethrin	Pyrethroid	WHO tube	Resistant
Permethrin	Pyrethroid	WHO tube	Resistant
Prallethrin	Pyrethroid	Bottle	Resistant
Transfluthrin	Pyrethroid	Bottle	Resistant
Malathion	Organophosphate	WHO tube	Possible resistance
Imidacloprid	Neonicotinoid	Bottle	Susceptible
Flupyradifurone	Butenolide	Bottle	Susceptible

Classification follows WHO criteria: susceptible above 98% mortality at 24 h, possible resistance 90 to 98%, resistant below 90% (World Health Organization 2022).

4 Discussion

Of the four actions added to the routine programme, only extra container elimination with Bti was followed by a substantial fall in egg density, and even that could not be confirmed statistically. Zone 5 ended 47% below the control zone's trajectory. Once the correlation between successive rounds is allowed for, the interval runs from a 75% reduction to a 13% increase, and part of the decline began before the change. We read it as a signal worth testing, not as an established effect.

For a municipality whose routine is running and whose counts are not falling, the practical question is which addition to try first. The ranking here is consistent: larval work with Bti at the top (0.53), clean-ups and larval work with spraying in the middle (0.87 and 0.92), and ULV alone at the bottom (1.12). None of the direct comparisons between actions reached significance, so the ranking is a hypothesis rather than a finding. It is also the ranking the wider evidence predicts. Because each action was confined to the blocks around high-count traps, it asked agents for extra work in a few places rather than across a whole zone.

If the zone 5 signal is real, its mechanism is plausible. Removing or treating containers stops adults emerging, and the effect accumulates across generations instead of dissipating with the aerosol. The heat maps fit that reading: density did not fall in the fortnight after an action but drifted down over successive rounds, although the event study shows the drift had begun earlier. Because elimination and Bti were applied together, the design cannot say which of them, or their combination, mattered. Systematic reviews of Bti for dengue vectors report reductions of this order when coverage is adequate (Boyce et al. 2013), and the integrated programmes that achieved sustained suppression in Brazilian cities were built on larval habitat management (Regis et al. 2013).

The spraying estimates agree with reviews of peridomestic space spraying, which have found little consistent effect on dengue vectors for fifteen years (Esu et al. 2010; Bonds 2012). Adding ULV to the zone 5 action did not improve on it. The direct contrast between zones 2 and 5 was 1.75 (0.77–3.97), so no additional benefit was detected at this study's precision, and a harm of that size cannot be excluded either.

Several explanations are available. *Ae. aegypti* rests indoors, in bedrooms and bathrooms, where ULV droplets from the street arrive in small quantities. The population we sampled was resistant to all four pyrethroids tested, including the prallethrin in the product sprayed, which matches the picture across Brazil (Valle et al. 2019; Melo Costa et al. 2020; Moyes et al. 2017). Nationwide surveys have documented the same pat-tern for the larvicide temephos (Chediak et al. 2016) and for adulticides at the state level (Hayd et al. 2020). Imidacloprid, the other active ingredient, retained full activ-ity in the laboratory. This study cannot separate resistance from indoor resting, spray coverage or dose as the reason spraying showed no effect.

Clean-up campaigns present a subtler case. Zone 8 showed a moderate point estimate (RR 0.87) that did not reach significance, and its northern sector stayed dense throughout. That zone holds most of the city's strategic points, and scrapyards generate containers faster than four-weekly drives remove them. There may also be a measurement effect. When productive containers disappear, gravid females fall back on whatever suitable sites remain, and an ovitrap is an excellent one (Abreu et al. 2015; Reiter 2007). A rise in trap counts after large containers are removed can therefore reflect redistribution of oviposition rather than a larger population. This ambiguity is intrinsic to using ovitraps to evaluate source reduction and deserves explicit attention in future designs.

Rainfall accounted for more deviance than the four actions together, and minimum temperature was also associated with egg density. Neither finding is new (Canyon et al. 1999; Maciel-de Freitas et al. 2007; Kolimenakis et al. 2021), but the comparison is instructive: in these data, a wet fortnight moved egg density more than any control action did. It also argues for keeping weather in any evaluation model, since a mild intervention effect and a wet season can otherwise be confused.

Zones 2 and 11 raise a design point. Both began with roughly 20 eggs per trap examined against 50 in the other three, and both were assigned ULV. With so little density to remove, either would need a large sample to detect a moderate effect, and their confidence intervals are correspondingly wide. We cannot exclude a modest benefit from ULV in zone 2 or a modest harm in zone 11. What we can say is that neither approached zone 5's point estimate.

4.1 Limitations

This is exploratory field evidence from one city. Packages were allocated to zones without randomisation, and one zone per action means zone-level confounding cannot be separated from the effect of the action. Zone 8's strategic points and zone 6's vertical housing are two obvious examples. The difference-in-differences design controls for anything shared across zones over time, such as the season and the dengue epidemic, but not for a zone-specific shock coinciding with the intervention period. A replicated design needs several zones per action, allocated at random or matched on baseline egg density.

The model has 245 zone-rounds but only five zones. Autocorrelation-consistent errors deal with correlation within a zone over time; nothing short of more zones deals with the small number of units. Four actions were tested, and after Holm adjustment none comes near significance.

Egg density is an indirect measure. It tracks adult population fluctuations well (Codec¸o et al. 2015) and correlates with local case distribution (de Albuquerque et al. 2018), but it is not adult abundance and is not transmission. Ovitrap counts also depend on how many competing breeding sites remain, so removing containers can shift oviposition into the traps (see Discussion). Eggs were not identified to species. We did not measure dengue incidence as an outcome, and this study was not powered to. Intervention coverage was recorded as delivered rather than measured. We know actions were carried out within the 150 m radius; we do not know what fraction of premises inside that radius was actually entered. The additional visits were entered in SisPNCD together with routine ones, so their number, and the share of premises closed or refused among them, cannot be separated out. The same holds for the containers they eliminated or treated. The focal-treatment counts in Section 2.4 show that larval work rose more in the zones meant to receive it, not how much of it the ovitrap trigger added. Differences in real coverage between zones would show up here as differences in effect.

Susceptibility was measured once, on paddles pooled from the five zones, so it characterises the population sampled on that occasion and cannot show whether resistance differed between zones.

Finally, only zone 6 served as control, and its own indices moved over the study. Its positivity rose while its MOI fell slightly, which is consistent with eggs spread across more traps rather than a real change in population size.

4.2 Implications for practice

Ovitraps did the first job asked of them, saying where to send agents, at a realistic operational load: about 100 traps per round and a loss rate under 3% for a municipality of 159,000 people, with the heat maps drawn by the platform. The second job, judging afterwards whether it had been worth it, needs more zones than one city's study provided. With five, fortnightly counts picked up the season and the rain clearly but could not confirm a 47% difference between zones.

The direction of the estimates is consistent. Where a municipality running the routine programme sees counts that do not fall, the results from this city favour trying extra container elimination with focal Bti in the blocks the traps flag before adding spraying. The absence of any detectable benefit from ULV, in a population resistant to its pyrethroid component, gives no entomological support for it here. We did not measure costs, so we make no claim about cost-effectiveness. Susceptibility to imidacloprid and flupyradifurone leaves room for rotation (World Health Organization 2022), but rotation addresses the compound, not the delivery problem.

5 Conclusion

Over two years in Patos de Minas, ovitrap egg density directed and evaluated four actions added to the routine programme. Extra container elimination with focal Bti gave the largest relative reduction, 47%, but the estimate was imprecise, partly pre-ceded the change, and did not reach significance. Clean-up campaigns and larval work combined with ULV sat close to no effect, and ULV alone had the least favourable

estimate, in a population resistant to all four pyrethroids tested. Rainfall moved egg density more than any additional action. A trial with several zones per action is the next step.

Statements and Declarations

Funding. The authors declare that no specific funding was received for this work. Field activities were carried out as part of the routine *Aedes* control programme of the municipal health department of Patos de Minas.

Competing interests. The authors declare no competing interests.

Author contributions. DCN designed and coordinated the field study, supervised ovitrap deployment and egg counting, and drafted the manuscript. LDA carried out the statistical analysis and prepared the figures. JBPL conceived and supervised the study and the insecticide susceptibility bioassays. All authors read and approved the final manuscript.

Ethics approval. The study used secondary entomological data without personal information and applied the methods already in routine use by the municipal *Ae. aegypti* control programme, and therefore did not require review by a human research ethics committee under Brazilian National Health Council rules.

Consent to participate. Residents of the premises where ovitraps were installed signed an informed consent form.

Consent for publication. Not applicable.

Data availability. The fortnightly ovitrap counts and derived indices analysed in this study, together with the paired meteorological series, are provided as supplementary material, with the Python scripts that reproduce every table and figure. Georeferenced trap records are held in the Conta Ovos platform (https://contaovos.com/pt-br/) and in SisPNCD, and are available from the municipal health department of Patos de Minas on reasonable request.

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