First National-Scale Evaluation of Temephos Resistance in *Aedes Aegypti* in Peru.

Miriam Palomino (✉ mpalominosal@gmail.com)

Fundação Oswaldo Cruz: Fundacao Oswaldo Cruz

Jesus Pinto

Instituto Nacional de Salud

Pamela Yañez

Instituto Nacional de Salud

Anali Cornelio

Instituto Nacional de Salud

Luciana Dias

Fundacao Oswaldo Cruz

Quesia Amorim

Fundacao Oswaldo Cruz

Audrey Lenhart

Centers for Disease Control and Prevention

Jose Bento Pereira Lima

Fundacao Oswaldo Cruz

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Abstract

Background: Resistance of *Aedes aegypti* to insecticides can lead to operational failures in control programs. Knowledge of the spatial and temporal trends of this resistance is needed for effective monitoring, on which vector control decision-making should be based.

Methods: The organophosphate insecticide temephos was evaluated in L3 larvae in dose-response bioassays with eleven concentrations of the insecticide, following WHO recommendations. The mosquitoes tested were *Ae. aegypti* larvae of F1 and F2 generations from eggs collected with ovitraps during 2018 and 2019.

Results: The results segregated into two geographic groups. The first group, from the coast, was mostly resistant to temephos ($RR > 10$), with three populations (AG, CR and LO) showing RR values greater than 20 (AG 21.5, CR 23.1, LO 39.4). The populations in the second group, from the jungle of the Amazon and high jungle, showed moderate levels of resistance (defined as RR values between 5 and 10) with values ranging between 5.1 in JN and 7.1 in PU. Exception in this region was the population of PM, which showed a RR value of 28.8 to this insecticide.

Conclusions: The results demonstrate that *Ae. aegypti* populations in Peru present different resistance intensities to temephos, even after the suspension of its widespread use. Resistance to this larvicide should continue to be monitored because it is possible that resistance to temephos could decrease in the absence of routine selection pressures.

Background

Aedes aegypti is the main vector of arthropod-borne viral infections transmitted to humans such as dengue, yellow fever, chikungunya and Zika [1, 2]. The vector is present in tropical and subtropical regions of Southeast Asia, the Pacific and the Americas, where these viruses also circulate. In these places there are local spatial variations in the presence and density of the vector that are strongly influenced by rainfall, temperature and degree of urbanization [3]. It has been observed that this mosquito has now adapted to areas at higher altitudes than its traditional range, such as in Cochabamba (Bolivia) at 2550 m.a.s.l. [4] and in Bello (Colombia) at 2302 m.a.s.l. [5], and has colonized places such as Ica (Peru), which has an arid temperate climate with an annual rainfall of only 2 mm [6, 7]. These adaptations have led to a wide dispersal of the vector, and with it, the presence of arboviruses that often follow the same pattern of dispersal.

It is estimated that about 2.5 billion people, representing 40% of the human population worldwide, live in areas at risk of dengue transmission [8], and there are an estimated 390 million cases of dengue per year in tropical and subtropical areas [3]. In addition, in the last five years, *Ae. aegypti* has been responsible for the spread of chikungunya and Zika to the region of the Americas, causing significant stress to health systems, as well as social and economic disruption [9].

In Peru, the only vector of dengue, chikungunya and Zika is *Ae. aegypti*, which after being locally eradicated in 1958, recolonized the country in 1984 [10]. The first outbreak of dengue occurred six years later due to dengue virus serotype 1 (DENV-1) [11]. Subsequently, low-incidence outbreaks occurred until 2001, when a major outbreak occurred with 23,304 cases, confirming the circulation of the four serotypes of dengue virus (DENV) [12]. In the years 2017 and 2020, significant outbreaks occurred with 68,290 cases and 52,826 cases, respectively [13]. The first reported cases of chikungunya and Zika occurred in 2015 and 2016, respectively [14, 15].

In Peru, *Aedes*-borne arboviruses are present in three ecological regions: the coast, the jungle of the Amazon and the Andes Mountains [16, 17]. However, this last region presents a diversity of altitudes that is differentiated into two areas: the Andean highlands (altitudes greater than 2300 m.a.s.l.) and high jungle (400 to 1400 m.a.s.l.; located on the eastern flank of the Andes) [18], with the *Aedes*-borne arboviruses only affecting areas of lower altitude. *Aedes aegypti* are widely distributed in 21 of Peru's 24 departments and the constitutional province of Callao, and have been identified in

527 districts [19], where approximately 22 million people live, putting 70.4% of the Peruvian population at risk of contracting arboviruses transmitted by this vector.

The main dengue control strategy implemented in Peru until 2016 was focal treatment of larval habitats with the organophosphate (OP) insecticide temephos because of its easy dosage, application and acceptability by the community. Likewise, the adulticides used in the 1990s were the OPs fenitrothion and malathion [20]. Since the 2000s, pyrethroid (PY) insecticides have been used (cyfluthrin, deltamethrin, alpha-cypermethrin and cypermethrin) [16, 21], applied using thermal and cold fog equipment that can be manually carried or mounted on trucks. However, in 2015, Pinto et al. [22] detected resistance to PYs associated with knockdown resistance (*kdr*) mutations (Phe1534Cys, Val1016Ile), after which the Peruvian Ministry of Health (MINSA) established the rotation of PYs with the OP malathion. For larval control, the OP temephos was switched with the insect growth regulator (IGR) pyriproxyfen because temephos shares the same mode of action as the OP malathion. The switch away from using temephos, however, was made without knowing the susceptibility status of *Ae. aegypti* populations to this insecticide.

Surveillance of insecticide resistance in arthropod vectors in Peru is an activity that falls under the Instituto Nacional de Salud (INS) and Regional Reference Laboratories (LRRs), which in the context of a decentralized health system requires coordinated work. However, many of the LRRs do not have entomology laboratories or insectaries to routinely carry out the various roles and responsibilities [21, 53]. This situation results in the weakening of the vector surveillance and control programs, despite ongoing increases in the numbers of cases of *Aedes*-borne arboviruses. The continuous use of insecticides results in selective pressures that result in physiological or behavioural adaptation, a phenomenon known as resistance [54]. Insecticide resistance can lead to operational failures, requiring rotations of insecticides with different modes of action or mosaic treatments to manage insecticide resistance [36]. Ideally, these strategies should be carried out pre-emptively to preserve insecticide efficacy but also reactively to mitigate or reverse resistance [54].

This study aimed to determine the levels of resistance to temephos in 39 Peruvian populations of *Ae. aegypti* from the three ecological regions of Peru after having used temephos for more than 25 years, following an interruption of three years.

Methods

Sampling and Study Area

We tested the F1 or F2 generations of *Ae. aegypti* from colonies maintained at the Peruvian National Institute of Health (INS), which were established from eggs collected in 39 localities in the departments of Tumbes, Piura, La Libertad, San Martín, Loreto, Ucayali, Junin and Madre de Dios during April 2018 and January 2019 (Figure 1). The colonies were obtained from eggs collected with ovitraps, which were distributed every 200 linear meters covering the urban area of the locality, according to the parameters established by MINSA [23]. The ovitraps were installed in intra- and peri-domestic areas, containing a strip of paper towel as oviposition substrate [23, 24] and 10% hay infusion as attractant [25]. The localities sampled are located in three ecological regions showing distinct weather patterns [6], variations in the incidence of dengue [16, 17] and differing insecticide resistance profiles [22].

Mosquito collection and laboratory rearing

The paper strips containing eggs were left to dry for one week and then soaked in water to hatch the F0 generation [26]. The F1 and F2 generations were subsequently established in the insectary of the Laboratorio de Referencia Nacional de Entomología (LRNE), from Centro Nacional de Salud Pública, INS, Lima, Peru (Table 1). Humidity and temperature conditions in the insectary were maintained at 70±10% RH and 26±2°C, respectively. *Aedes aegypti* of the susceptible Rockefeller reference strain were used as a control for the resistance tests [27].

Table 1
Aedes aegypti populations used in all tests.

Locality / Population	Code	Collection date	N° Collected eggs	Generation evaluated
Aguas Verdes	AG	Apr, 18	1148	F1
Zarumilla	ZA	Apr, 18	1025	F1
Sagaro	SA	Apr, 18	748	F1
Pampa Grande	PG	Apr, 18	2448	F1
Corrales	CR	Apr, 18	833	F1
Cabuyal	CB	Apr, 18	233	F1
La Cruz	CZ	Apr, 18	187	F1
Mancora	MA	Apr-May, 18	2051	F1
Los Órganos	LO	Apr-May, 18	1573	F1
Bellavista	BE	Apr-May, 18	1992	F1
Comunidad Saludable	CS	Apr-May, 18	515	F1
Micaela Bastidas	MI	Apr-May, 18	468	F1
San José	JO	Apr-May, 18	1062	F1
Tambogrande	TA	Apr, 18	1360	F1
Chulucanas	CH	Apr-May, 18	2398	F1
La Esperanza	ES	Apr, 18	4580	F1
El Porvenir	PO	Apr, 18	3023	F1
Florencia de Mora	FL	Apr, 18	1673	F1
Laredo	LA	May, 18	2442	F1
Chao	CA	Apr, 18	1507	F1
Virú	VI	Apr, 18	781	F1
Moyobamba	MO	Nov-Dec, 18	n.a	F2
Morales	MR	Nov, 18	900	F1
Banda de Shilcayo	BS	Nov, 18	n.a	F2
Juanjui	JJ	Dec, 18	1846	F1
Nuevo Bambamarca	BM	Nov, 18	n.a	F2
Tocache	TO	Nov, 18	n.a	F2
Satipo	ST	Sep, 18	1216	F1
Iquitos	IQ	Jun, 18	6651	F1
Punchana	PU	Jun, 18	733	F1
Belén	BN	Jun, 18	775	F1

Locality / Population	Code	Collection date	N° Collected eggs	Generation evaluated
San Juan Bautista	JN	Jun, 18	1876	F1
San José	SY	Aug, 18	324	F1
Yarinacocha	YA	Aug, 18	2342	F1
Callería	PL	Aug, 18	2123	F1
José Carlos Mariátegui	PA	Aug, 18	1487	F1
Manantay	MY	Aug, 18	1391	F1
San Fernando	FE	Aug, 18	562	F1
Puerto Maldonado	PM	Jan, 19	891	F1

Larvicide Susceptibility Testing

Dose-response susceptibility tests were performed with temephos following WHO recommendations [28]. The larvae were exposed to a wide range of the insecticide concentrations in order to evaluate the larvicidal activity and thereby determine the values of the lethal concentration (LC) LC_{50} and LC_{95} for each study population. Four replicates per concentration, and eleven concentrations were evaluated with 20 3rd -stage larvae (L3) in 100 mL of insecticide solution per replicate. Insecticide solutions were prepared with temephos active ingredient (Chem Service, West Chester, USA), using a concentration range of 0.004 to 0.324 mg/mL for field populations, and 0.002 to 0.012 mg/mL for the Rockefeller strain. Simultaneously, a control group with 4 replicates exposed only to 600 μ L of ethanol solvent was evaluated [28]. Each test was carried out on three different days to ensure the reproducibility of the method and consistency of the results [28, 29].

Data Analysis

The values for LC_{50} and LC_{95} were calculated from a log dosage-probit mortality regression line using probit analysis, Polo-PC statistics package [30] for each population. Resistance ratios (RRs) were also calculated to define the intensity of resistance in the field populations. To calculate the RR_{50} ($i=50$) and the RR_{95} ($i=95$) equation 1 was used:

$$RR_i = \frac{LC_{i(field)}}{LC_{i(RockefellerLineage)}} \dots \text{Equation 1}$$

The population was considered susceptible when the $RR < 5$; when the RR was between 5 and 10, the population was considered to have moderate resistance; and when the $RR \geq 10$, the population was considered highly resistant.

Results

Thirty-nine mosquito populations were obtained from localities with different incidence of arboviruses and located in three ecological regions of Peru. The F1 and F2 generations were evaluated and a total of 37,440 L3 larvae were tested across all bioassays.

Table 2 and Figure 2 show the values of the LC_{50} and LC_{95} , the resistance ratios (RR_{50} and RR_{95}) and the slope of the probit regression lines for the insecticide temephos in the 39 populations of *Ae. aegypti*. The differences between the RR_{95} of all populations were analysed according to the criteria of Mazzarri and Georghiou [31] and Sá et al. [29], and showed that the three regions could be grouped into two groups: coastal and jungle (including the Amazon and high jungle). In the coastal group, the populations were mostly highly resistant to temephos ($RR > 10$), with three populations

showing values greater than 20 (AG 21.5, CR 23.1, LO 39.4). Some populations (PG, MI, TA, BE, CS, ES and LA) showed moderate levels of resistance ($5 \leq RR < 10$) and only four populations (JO, CH, CA and VI) were considered susceptible ($RR < 5$). In the jungle group, most of the populations showed moderate levels of resistance ($5 \leq RR < 10$) with values between 5.1 in JN and 7.1 in PU, while some populations were considered susceptible ($RR < 5$) with values between 2.2 in MO and 4.9 in YA and PA, with the notable exception of the population of PM, which was considered highly resistant with a RR value of 28.8.

Table 2

Temephos susceptibility profiles of Peruvian populations showing slope values, Lethal Concentration values (LC), and Resistance Ratios (RR).

Department	Lineage / Population	Code	F	N	LC (95% CI)					RR	
					Slope	LC50		LC95		RR ₅₀	RR ₉₅
	Rockefeller			2880	5.67	0.005	(0.004-0.006)	0.01	(0.009-0.011)	1.0	1.0
Tumbes	Aguas Verdes	AG	F1	2880	2.50	0.047	(0.045-0.050)	0.215	(0.193-0.245)	9.4	21.5
	Zarumilla	ZA	F1	2880	3.22	0.036	(0.034-0.037)	0.116	(0.108-0.127)	7.2	11.6
	Sagaro	SA	F1	2880	3.65	0.039	(0.038-0.041)	0.110	(0.103-0.119)	7.8	11.0
	Pampa Grande	PG	F1	2880	3.72	0.032	(0.031-0.033)	0.089	(0.083-0.095)	6.4	8.9
	Corrales	CR	F1	2880	2.28	0.044	(0.041-0.046)	0.231	(0.201-0.273)	8.8	23.1
	Cabuyal	CB	F1	2880	2.42	0.028	(0.026-0.030)	0.135	(0.122-0.152)	5.6	13.5
	La Cruz	CZ	F1	2880	3.74	0.056	(0.054-0.058)	0.154	(0.145-0.166)	11.2	15.4
Piura	Mancora	MA	F1	2880	3.7	0.071	(0.069-0.074)	0.199	(0.181-0.222)	14.2	19.9
	Los Organos	LO	F1	2880	3.01	0.111	(0.090-0.136)	0.394	(0.321-0.484)	22.2	39.4
	Bellavista	BE	F1	2880	3.05	0.025	(0.024-0.026)	0.086	(0.080-0.094)	5.0	8.6
	Comunidad Saludable	CS	F1	2880	2.75	0.024	(0.022-0.026)	0.094	(0.087-0.103)	4.8	9.4
	Micaela Bastidas	MI	F1	2880	3.36	0.020	(0.018-0.021)	0.060	(0.057-0.065)	4.0	6.0
	San Jose	JO	F1	2880	3.88	0.016	(0.015-0.016)	0.041	(0.039-0.045)	3.2	4.1
	Tambogrande	TA	F1	2880	4.58	0.025	(0.024-0.026)	0.057	(0.054-0.060)	5.0	5.7
	Chulucanas	CH	F1	2880	4.02	0.015	(0.014-0.015)	0.038	(0.036-0.041)	3.0	3.8
La Libertad	La Esperanza	ES	F1	2880	2.54	0.018	(0.016-0.019)	0.078	(0.071-0.087)	3.6	7.8
	El Porvenir	PO	F1	2880	2.59	0.025	(0.023-0.026)	0.107	(0.097-0.119)	5.0	10.7
	Florencia de Mora	FL	F1	2880	2.81	0.029	(0.028-0.031)	0.113	(0.103-0.125)	5.8	11.3

Department	Lineage / Population	Code	F	N	LC (95% CI)					RR	
					Slope	LC50		LC95		RR ₅₀	RR ₉₅
	Laredo	LA	F1	2880	3.37	0.021	(0.020-0.022)	0.066	(0.061-0.071)	4.2	6.6
	Chao	CA	F1	2880	7.18	0.010	(0.010-0.010)	0.021	(0.020-0.023)	2.0	2.1
	Viru	VI	F1	2880	5.63	0.011	(0.011-0.012)	0.022	(0.022-0.024)	2.2	2.2
San Martín	Moyobamba	MO	F2	2880	4.39	0.009	(0.009-0.010)	0.022	(0.021-0.024)	1.8	2.2
	Morales	MR	F1	2880	4.00	0.021	(0.021-0.022)	0.055	(0.052-0.059)	4.2	5.5
	Banda de Shilcayo	BS	F2	2880	4.33	0.023	(0.022-0.024)	0.055	(0.053-0.059)	4.6	5.5
	Juanjui	JJ	F1	2880	3.65	0.010	(0.009-0.010)	0.042	(0.039-0.047)	2.0	4.2
	Nuevo Bambamarca	BM	F2	2880	4.85	0.011	(0.010-0.011)	0.023	(0.022-0.025)	2.2	2.3
	Tocache	TO	F2	2880	4.14	0.010	(0.009-0.010)	0.024	(0.023-0.026)	2.0	2.4
Junin	Satipo	ST	F1	2880	3.45	0.019	(0.018-0.020)	0.058	(0.054-0.062)	3.8	5.8
Loreto	Iquitos	IQ	F1	2880	4.02	0.026	(0.025-0.027)	0.067	(0.063-0.071)	5.2	6.7
	Punchana	PU	F1	2880	4.73	0.032	(0.031-0.033)	0.071	(0.068-0.075)	6.4	7.1
	Belen	BN	F1	2880	4.36	0.023	(0.022-0.024)	0.054	(0.052-0.057)	4.6	5.4
	San Juan Bautista	JN	F1	2880	4.18	0.021	(0.020-0.022)	0.051	(0.048-0.054)	4.2	5.1
Ucayali	San Jose-Yarinacocha	SY	F1	2880	3.89	0.017	(0.017-0.018)	0.069	(0.063-0.076)	3.4	6.9
	Yarinacocha	YA	F1	2880	4.21	0.02	(0.019-0.020)	0.049	(0.046-0.052)	4.0	4.9
	Callería	PL	F1	2880	4.02	0.015	(0.014-0.015)	0.038	(0.036-0.041)	3.0	3.8
	José Carlos Mariátegui	PA	F1	2880	3.83	0.018	(0.018-0.019)	0.049	(0.046-0.053)	3.6	4.9
	Manantay	MY	F1	2880	3.58	0.018	(0.017-0.019)	0.052	(0.048-0.056)	3.6	5.2
	San Fernando	FE	F1	2880	5.07	0.015	(0.014-0.015)	0.031	(0.030-0.033)	3.0	3.1

Department	Lineage / Population	Code	F	N	LC (95% CI)					RR	
					Slope	LC50		LC95		RR ₅₀	RR ₉₅
Madre de Dios	Puerto Maldonado	PM	F1	2880	2.11	0.048	(0.045-0.051)	0.288	(0.243-0.355)	9.6	28.8

However, the resistance ratios of the populations were heterogeneous even within the same department. In Tumbes, for example, most populations were highly resistant to temephos ($RR > 10$), except for the PG population ($RR=8.9$) which showed moderate resistance to this insecticide. On the other hand, Piura had two populations (MA and LO) that exhibited high RR values ($RR > 19$), but also had two susceptible populations with low values ($RR < 5$). In La Libertad, the resistance ratios varied between 2.1 in CA to 11.3 in FL, showing that this department also presented heterogeneous resistance ratios, but with two populations where the lowest values were detected ($RR < 2.5$). The departments of San Martin, Junin, Loreto and Ucayali were more homogeneous, with values between 2.2 in MO and 7.1 in PU. However, despite the generally lower RR values in the jungle areas, in the jungle department of Madre de Dios, the PM population showed a RR value of 28.8. However, the resistance ratios of the populations were heterogenous even within in the same locality. Populations (AG, ZA, SA, PG, CR, CB, PO, FL and PM) with the moderate level of resistance ($5 \leq RR < 10$) in RR_{50} but showed highly resistant to temephos ($RR > 10$) in RR_{95} and populations (CS, MI, ES, LA, MR, BS, ST, BN, JN, SY and MY) with low values in RR_{50} but showed moderate resistance in RR_{95} . Populations CZ, MA and LO, due to their highly resistant to temephos were more homogeneous, with high values of RR_{50} (11.2, 14.2 and 22.2) and RR_{95} (15.4, 19.9 and 39.4). Some populations (PG, BE, TA, IQ and PU) showed moderate level of resistance ($5 \leq RR < 10$) and 12 populations (JO, CH, CA, VI, MO, JJ, BM, TO, YA, PL, PA and FE), were considered more susceptible ($RR < 5$) in both RR_{50} and RR_{95} . In general, jungle localities were more homogeneous.

In Table 2 and Figure 3, the slope values of the probit regression lines of the field populations were lower than those obtained with the Rockefeller lineage, except in the CA population (slope=7.18), confirming the heterogeneity of temephos resistance in the field strains in relation to the reference strain.

Discussion

As part of a national strategic plan against dengue, chikungunya and Zika, a national survey to ascertain the susceptibility of *Ae. aegypti* to temephos was undertaken. The Peruvian INS, in coordination with Regional Health Directorates, surveyed 39 dengue endemic localities from April 2018 to January 2019. Overall, Peruvian populations of *Ae. aegypti* showed variable levels of resistance to temephos. According to the results these populations were clustered into two groups: 1) coastal populations with moderate to high resistance to temephos, and 2) jungle populations, which showed moderate resistance and some susceptibility, except for the Puerto Maldonado population which was highly resistant.

Higher levels of resistance to temephos were detected in coastal populations than in jungle populations, despite the fact that the northern coast was recolonized by *Ae. aegypti* ten years after the Peruvian Amazon [32, 33]. The reasons underlying this difference in susceptibility are likely diverse. For example, the departments along the northern coast of Peru were the most affected by the El Niño climatic phenomenon (1997-1998), with heavy rains that caused an increase in malaria and dengue cases [34, 35]. This situation led to the establishment of prevention and control activities, including insecticide space spraying and indoor residual spraying, mapping and treatment of larval habitats (including chemical control with temephos), and campaigns to eliminate potential larval habitats. These activities were carried out periodically in 333 localities in the departments of Tumbes, Piura, Lambayeque, and La Libertad, with the goal of eliminating larval habitats in urban, peri-urban, and rural areas. In addition, there is a growing urban concentration on the

coast, without planning or organization and with insufficient basic sanitation, due to constant migration from other regions due to violence, lack of job opportunities, education, access to technological resources, and poverty [35]. This rapid, unplanned urbanization favors the introduction and establishment of *Ae. aegypti*. However, the Peruvian coastline is formed by a desert strip interrupted by valleys whose main characteristic is the scarcity of rainfall [6], which should serve as a limiting factor for the transmission of arboviruses in this region with the important exception of the north coast, which experiences high temperatures and rainfall in the summer due to its proximity to the equator [17]. However, the potential benefit of this arid climate is overcome by the presence of larval habitats inside homes as a consequence of inadequate water storage due to deficient piped water supply in urban centers [16, 36]. Evidence of this can be seen in the types of larval habitats that predominate in and around homes, including water storage drums, cylinders, wells and flower vases [7]. As a result, access, availability and quality of water is an environmental determinant that creates conditions for the proliferation of vectors and transmission of arboviruses [37], as demonstrated by the Ministry of Health of Peru, who reported in 2016 that inadequate access to water was associated with 41.2% of cases of dengue [38].

The Peruvian Amazon presents different ecosystems than those seen on the coast. These jungle areas typically include humid and rainy tropical forests [6]. In this region, due to the constant rainfall, there is a greater abundance of potential larval habitats, especially those known as "los inservibles" (discarded objects, passively filled with rainwater). Unlike along the coast, these larval habitats are typically peri-domestic and unintentionally hold water [39]. In contrast to the urbanization along the coast, the jungle is the least populated region of the country and accounts for only 10% of the national population, and the infrastructure of Amazonian villages often reflects inadequate access to basic services, education and health [40]. The city of Puerto Maldonado is the capital of the Amazonian department of Madre de Dios, located in the southeastern part of the country. In this city, the *Ae. aegypti* population was the only one in the Peruvian Amazon that presented high resistance to temephos. The vector was introduced in this city in 1999 [41], fifteen years after the first record in the Peruvian jungle in 1984 [10]. Dengue cases were sporadic in the period from 2000 to 2016, with only one major outbreak reported in 2010 with 2952 recorded cases [42]. This low burden of dengue is supported by the findings of Salmón-Mulanovich et al. [43], who found low seroprevalence to DENV in a retrospective study conducted in Puerto Maldonado in 2018. This low disease burden suggests that vector control activities were not routine or intense, and that the local *Ae. aegypti* population has not experienced strong selection pressure with the larvicide temephos. The explanation for the high temephos resistance detected in this study could be due to the fact that the vector has spread across the border from Brazil and Bolivia. On the Brazilian side, the *Ae. aegypti* population from Rio Branco (Acre State) is highly resistant to temephos [44] and similarly, on the Bolivian side, the *Ae. aegypti* population in the border city of Cobija (department of Pando) has moderate resistance to temephos [45].

It is also important to consider that at the national level, the use of pesticides in the agricultural sector in Peru is intense, with the most widely used insecticides being organophosphates (OPs) [46]. Historically, organochlorines (OCs) were used between 1940-1950 (dichloro diphenyl trichloroethane [DDT], benzene hexachloride [BHC] and toxaphene) and the OPs parathion and methamidophos were introduced in 1950 and were used for several decades, with parathion used until 2005 [46] and methamidophos until 2018-19 [47]. In the 2000s, pyrethroids (deltamethrin, cypermethrin and alpha-cypermethrin) and carbamates (CA) (carbofuran, methomyl, carbosulfan and carbaryl) were introduced [46, 47]. In a study conducted in 2012, it was reported that 43% of farmers preferred to use OPs because they had a broad spectrum of action (contact, ingestion and fumigant effects) [48]. It is important to note that these pesticides were widely used on cotton, corn and potato crops in the northern and central coastal valleys, as well as in the Andes Mountains. Another important consideration is the illegal trade of pesticides through smuggling (mainly on the northern border), street sales, adulteration and counterfeiting of products, especially in the northern coast, central and southern parts of the Andes Mountains [49]. The continuous use of pesticides in agriculture has led to pest resistance to OCs and OPs due to misuse of pesticides and lack of pesticide management [46]. The agricultural sector is using the same classes of insecticides

that are being used for public health, so mosquitoes and other non-target insects may experience pressure from insecticides used in agriculture, resulting in the selection of populations that exhibit resistance to multiple insecticides [50].

The results of the present investigation demonstrate that Peruvian *Ae. aegypti* populations display a diversity of resistance to temephos. These results are consistent with resistance patterns observed in other field populations that have had intense selection pressure with temephos [51]. In Peru, temephos had been used for more than 25 years and it is thus not surprising that in some areas, resistance to this insecticide has reached very high levels. However, it is not possible to estimate or quantify the evolution of this resistance due to the lack of baseline information, a fact that is corroborated by the absence of data from Peru in the findings of the review of resistance prepared by Moyes et al. [52]. Resistance to temephos has been reported worldwide and at high levels in Tamil Nadu (India) [55], Caldas (Colombia) [56], Pernambuco (Brazil) [57], Martinique [58] and Bahia (Brazil) [59]; at moderate levels as in Tocantins (Brazil) [29], Laos (Asia) [60], Paraná (Brazil) [61], Quindío (Colombia) [62], Delhi (India) [63], Sao Paulo and Northeast Region (Brazil) [64], and susceptibility has been reported in Malaysia [65], Santiago Island (Cape Verde) [66] and Phitsanulok province in Thailand [50].

Chemical control of *Ae. aegypti* with temephos in Peru was continuous until 2015 when it was replaced by pyriproxyfen [67]; this could explain the moderate and low levels of resistance found in the populations of Pampa Grande, Bellavista, and Comunidad Saludable among others. This suggests that resistance to temephos is unstable in the absence of continuous selection pressure; a fact that is corroborated by the findings of Wirth and Georgiou [51], who detected significant decreases in the levels of resistance to temephos in the British Virgin Islands (Tortola) from a RR of 46.8 in 1985 [24] to 12.1 in 1992-93 [68] and then to 6.3 in 1995-96 [69] after the interruption of its use for more than 10 years. Similarly, Conde et al. [56] observed in Colombia (Caldas) the reduction of temephos resistance from RRs of 13.27 and 11.48 in 2007 to 4.75 and 5.61 in 2011 after discontinuation of its use for four years. Similarly, Brazil (Juazeiro do Norte) [70] observed a decrease in resistance to temephos from a RR of 10.4 to 7.2 after 7 years when this larvicide was replaced by *Bacillus thuringiensis israelensis* (Bti) and Rahman et al. [71] observed in Rio de Janeiro in the municipality of Campos dos Goytacazes the reduction of temephos resistance from a RR of 7.8 in 2001 to 2.6 in 2016 after discontinuation of its use for 15 years and detected significant decrease in the levels of resistance to temephos in the municipality of Itaperona from a RR of 25.6 in 2011 to 7.3 in 2016 after this larvicide was substituted by IGR. Consequently, rotating to a new insecticide with a different mode of action could be advantageous for temephos resistance management. WHO recommends the following compounds as alternative larvicides: Bti, diflubenzuron, methoprene, novaluron, pyriproxyfen and spinosad.

Following the observed resistance of Peruvian *Ae. aegypti* to pyrethroids, the OP malathion is being reintroduced [67]. It is uncertain how long this insecticide will remain effective if resistance to temephos has already been demonstrated or if there is cross-resistance between temephos and malathion. Wirth and Georgiou [51] suggested that resistance to malathion did not increase significantly under selection pressure with temephos and that adulticides exerted lower selection pressure than larvicides [24]. If this is indeed the case, it is reasonable to believe that malathion may still be effective in Peru, which is important considering the few chemical options available in Peru for vector control.

An important limitation to this study was the lack of additional evaluations that would allow us to better understand the evolution of resistance to temephos, as well as the characterization of the observed resistance by molecular and enzymatic methods. On this last point, Rodriguez et al. [72] in a study with Latin American populations, found that a Peruvian *Ae. aegypti* population presented variations in the intensity of resistance to different OPs (temephos RR=30, malathion 1.5, fenthion 6.6, pirimiphos-methyl 10, fenitrothion 1.1 and chlorpyrifos 4.3), with elevated activity of esterases related to resistance to temephos, while mono-oxygenases were associated with resistance to pirimiphos-methyl and chlorpyrifos.

Conclusions

The results of this study demonstrate that *Ae. aegypti* populations in Peru have different levels of resistance to temephos, even after its widespread use was suspended, spanning the spectrum from susceptible to high levels of resistance. It is necessary to continue monitoring resistance to this larvicide to understand if in the absence of widespread use, susceptibility to temephos may be recovered in the future. The implementation of insecticide resistance management strategies is important to preserve the efficacy of insecticides used in public health, and it is necessary to carry out vector control with multisectoral components that can improve the effectiveness of the *Ae. aegypti* control program in Peru.

Declarations

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Authors' contributions

MP and JBP conceived and designed the study. MP, JP, PY and AC carried out the field work. MP, LD, QA, PY and AC carried out the laboratory work. MP and LD analyzed and interpreted the data. MP, AL and JBP wrote the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Map of the selected localities in the three ecological regions of Peru.

Figure 2

Temephos resistance ratios (RRs) in Peruvian populations of *Ae. aegypti* 2018-2019

Figure 3

Linear regression of *Aedes aegypti* mortality after exposure to the organophosphate temephos in Peruvian populations compared to the susceptible Rockefeller strain (in blue). A: The jungle of the Amazon, B: Coast and C: High jungle.