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Simple Summary: There are few reported about *Aedes albopictus* in Guatemala. The objective of this study was to describe the spatial distribution and breeding site of *Ae. albopictus* in the Department of Santa Rosa during the year 2024. The presence of the vector was identified in 12 (85.71%) of the 14 municipalities of the Department. 31.34% (21/67) of the samples were collected during the dry season and 60.66% (46/67) during the rainy season. Ten deposits tested were positive for *Ae. albopictus* larvae with predominance of artificial deposits of 61.19% (41/67), among them, tires (34.33%) and cans (16.42%) stand out. *Ae. albopictus* is described in the rural area of the majority of the municipalities of the Department with a variety of the breeding sites, mainly in the non-useful artificial deposits. A wider amount of studies about *Ae. albopictus* that provide information about the bionomy of the vector, its relation with viruses and the transmission of diseases are necessary.

Abstract: *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) is considered as vector of emerging arboviruses, such as dengue, chikungunya, zika, and yellow fever virus. Although this mosquito has been described in Guatemala since 1995, there are few reported about this species in the country. The objective of this study was to describe the spatial distribution and breeding site of *Ae. albopictus* in the Department of Santa Rosa during the year 2024. A cross-sectional study was performed during dry season (November-April) and rainy season (May-October) of the Santa Rosas' Department. The data analysis was performed with EPIDAT, version 4.2. 67 samples of *Ae. albopictus* were selected. The presence of the vector was identified in 12 (85.71%) of the 14 municipalities of the department. 31.34% (21/67) of the samples were collected during the dry season and 60.66% (46/67) during the rainy season. Ten deposits tested were positive for *Ae. albopictus* larvae with predominance of artificial deposits of 61.19% (41/67), among them, tires (34.33%) and cans (16.42%) stand out. *Ae. albopictus* is described in the rural area of the majority of the municipalities of the Department with a variety of the breeding sites, mainly in the non-useful artificial deposits. A wider amount of studies

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Keywords: *Aedes albopictus*; breeding sites; spatial distribution, non-useful artificial deposits; Guatemala

1. Introduction

Aedes albopictus (Skuse, 1894) also known as "Asian tiger" has generated great concern during the last years due to its fast propagation and easy adaptability in the environment [1,2]. Originary from southeast Asia, is currently considered as the most invasive mosquito in the world and it is reported as one of the vectors with a wide geographic expansion [3].

This insect transmits emerging arboviruses, such as dengue (DENV), chikungunya (CHIKV), zika (ZIKV), and yellow fever virus (YEV) [4-6]. In Europe, the presence of *Ae. albopictus* has not been common and it is involved in the transmission of dengue in countries such as France, Italy, and Spain [7-9]. Rey et al. [10] showed the pivotal role of "Asian Tiger" in the transmission of DENV in Colombia, Brazil, Costa Rica, and Mexico.

More evidence of the medical importance of this mosquito are exposed by García-Rejón et al. [11] These authors isolated and identified virus of eastern equine encephalitis, keystone, cache valley, la Crosse, west Nile, DENV (all the serotypes), YFV, ZIKV, and CHIKV in specimens of *Ae. albopictus.*

The "Asian Tiger" possesses a high efficiency to transmit DENV both horizontally and vertically, which transforms it into a silent reservoir of the virus, modifying the local epidemiological pattern of the disease [11]. In the same sense, this species can disseminate zoonotic viruses in urban and peri-urban environments from the wide range of animals from which it ingests blood [11]. In facts, investigation done in Brazil, where the detection of DENV-3 was made in the years during which no autochthonous human cases with this serotype were recorded [12]. Furthermore, YFV was isolated in *Ae. albopictus* females in the state of Rio de Janeiro, which could imply that this species is acting as an additional vector of disease with wild and rural cycles causing a possible bridge to the urban area [13]. In the same way, *Ae. albopictus* participates in the natural cycle of the horizontal transmission of DENV along with *Ae. aegypti* and could be useful as sentinel species to monitor the virus interepidemic periods [11].

In 1995, *Ae. albopictus* was reported for the first time in the Comunidad de Puerto Barrios in the Department of Izabal, Guatemala [14]. Since then, this species has been documented in 11 out of the 12 Departments of the country [15]. However, there are very few reported specifically in the Department of Santa Rosa. For this reason, the objective of this study is to describe the spatial distribution and breeding site of *Ae. albopticus* in the Department of Santa Rosa during 2024.

2. Materials and Methods

- Study Design

A cross-sectional study was performed during the dry season (November-April) and the rainy season (May-October) in the Department of Santa Rosa during 2024.

- Area of Study

The Department of Santa Rosa is located in the southeast region of Guatemala, being Cualiapa the main municipality. The coordinates of the location are: 14° 16′42′′ N, 90° 18′00′′ S. According to the census performed in 2024, the Department has an estimated population of 462, 019 inhabitants and a territory extension of 2, 955 km². Santa Rosa is bordered to the north with the Departments of Guatemala and Jalapa, to the east with Jutial, to the South with the Pacific Ocean, and to the West with the Department of Escuintla. (**Figure 1).**

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Figure 1. Localization and distribution of the 14 municipalities (represented with different colors) of the Department of Santa Rosa, Guatemala.

From: Map of the Department of Santa Rosa, Guatemala.

The geographic configuration is wide, with altitude ranging from 0 meters above sea level (m.a.s.l.) to 2,400 (m.a.s.l). The Department has a tropical savanna climate according to the Köppen classification [16]. (**Table 1**).

Table 1. Values of the temperature, precipitation and altitude for each municipality of the Department of Santa Rosa, Guatemala, 2024.

No	Municipalities	Average Maximum Temperature (^{0}C)	Average Minimum Temperature (^{0}C)	Average Temperature (^{0}C)	Average Precipitation (mm)	Average Height (msnm)
1	Cuilapa	21.83	8.92	16.08	107.82	818.50
2	Santa Rosa de Lima	33.85	13.28	22.17	102.27	1101.75
3	Nueva Santa Rosa	33.85	13.28	22.17	102.27	1083.00
4	San Rafael Las Flores	26.72	15.34	20.69	122.05	1414.67
5	Casillas	26.73	10.59	17.46	146.62	1225.20
6	Santa Cruz Naranjo	27.35	16.81	21.78	157.45	1200.00
7	Barberena	29.73	14.54	20.48	156.54	1183.67
8	Pueblo Nuevo Viñas	22.05	21.49	21.77	92.67	1250.00
9	Taxisco	34.36	23.32	28.01	169.59	420.00
10	Guazacapán	33.26	22.06	27.76	183.64	605.33
11	Chiquimulilla	34.13	23.40	28.02	109.58	504.67
12	San Juan Tecuaco	32.89	20.81	26.88	189.48	459.00
13	Santa María Ixhuatán	29.79	18.09	23.88	189.88	1047.00
14	Oratorio	31.93	19.84	25.79	174.32	737.25

Source: National Institute of Seismology, Volcanology, Meteorology, and Hydrology.

- Household inspection, collection, and identification of *Ae. albopictus* larvae.

The biological material was collected according to the norms and techniques established in the Operational Manual on Vector Surveillance and Control for Guatemala (MOVCVEG, Spanish acronyms) [17]. The surveys were conducted as part of the Entomological Vigilance during the dry season (November- April) and the rainy season (May-October). The deposits in the bedrooms, halls, yards, and gardens were carefully inspected.

The collection of larvae (only 4 instar, according to the MOVCVEG) was conducted with the use of Pasteur pipettes, flashlights or mirrors, and plastic trays. After that, the captured larvae were fixed in 70% ethanol in vials, to which labels were attached with the established model in the MOVCVEG that contains the following information:

Health Area (Department), Municipality, Locality (village, district, neighborhood, colony). Date of sample collection, deposit type (e.g. tires: artificial deposit), Location in the Household (intra or

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peridomicile), Utility of the Deposit (useful, not useful), Departmental Laboratory Diagnosis, Date of Diagnosis, Number of the Larvae and Pupae, Name of the Head of Household, Name of Collector.

The samples were transported to the Departmental Entomology Laboratory (LED) of the Vector-Borne Diseases Program of the Department of Santa Rosa. These were identified using stereoscopes (Olympus SZ2- ST, Japan) and Standard Taxonomic Key [18]. Once classified the samples, the results were put on the label and the referred data of each of them was transcribed into the Book of Entomological Samples. It is worth mentioning that 10% of the entomological samples registered and classified monthly at LED (randomly selected) are sent to the Taxonomy and Medical Entomology Laboratory of the Vector-Borne Diseases Program of the Ministry of Public Health and Social Assistance (MSPAS) of Guatemala for quality control.

- Sample Selection

✔ The population of study was confirmed by 81 mosquito samples recorded during the study period in the Entomological Sample Book, where the species *Ae. albopictus* was diagnosed.

Inclusion Criteria in the sample

✔ Samples testing positive with larvae *Ae. albopictus* or with coexistence of other mosquito populations.

Exclusion criteria

✔ Labeled samples that contain duplicated information

✔ Samples in which identification of the species is not allowed (fragmented or deteriorated larvae)

✔ Deposits only with pupae.

✔ Samples that present partial or total absence of the information or that it is unreadable.

- Classification of the deposits

Classification based on the type of reservoir [17,19]:

Useful Artificial Deposits (UAD): remain with water for human or animal consumption (e.g. barrels, water tanks, buckets, basins, and toilets).

Non-Useful Artificial Deposits (NUAD): they are found with or without water and have no use value for the inhabitant (e.g. bottles, cans, and tires).

Natural Deposits (ND): these are not man-made and can contain water (e.g. three hallows, solid cavities, coconut or egg shells, and plat axils).

- Classification according to the area

The information that defined an urban or rural area was taken from the classification performed by the Department of Surveillance and Control of Health Regulations (DVCRS) which belongs to the Departmental Directorate of Integrated Health Services Networks (DDRISS) of the Department of Santa Rosa.

- Data Analysis

A database was done with the application of Excel, version 2501 for Microsoft 365 and Google Earth with the information of the municipality, locality, and coordinates, which was incorporated to the Arcgis Program Pro 2023 for the elaboration of the spatial distribution map of *Ae. albopictus*. It used the EPIDAT Statistical Program, version 4.2 for the calculation of Absolute and Relative frequency, as well as percentage. The non-parametric Wilcoxon rank test with significance level $p<0.05$ was used for statistically significant differences between the number of deposits described as positive to *Ae. albopictus* in the rainy and dry season.

The databases of temperature and precipitation were taken from conventional and automatic stations of INSIVUMEH, (ICC) and (ANACAFÉ).

- Ethical Considerations

The Vector Coordinator of the Department of Santa Rosa, Mr. Joel Sarceño Cardona, permitted the use of the LED database. The samples were taken from the households by the vector's workers in compliance with their duties and obligations established in the MOVCVEG, after receiving the approval of the dwellers. The authors attest to the transparency and veracity of the data shown.

3. Results

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82% (67/81) of the total of the samples collected with *Ae. albopictus* diagnosis during the study period conforms to the samples of this investigation. These samples come from the 26 localities (24 rural and 2 urban) pertaining to 12 (85.71%) municipalities. In Pueblo Nuevo de Viña and Santa Cruz Naranjo no presence of the vector has been reported.

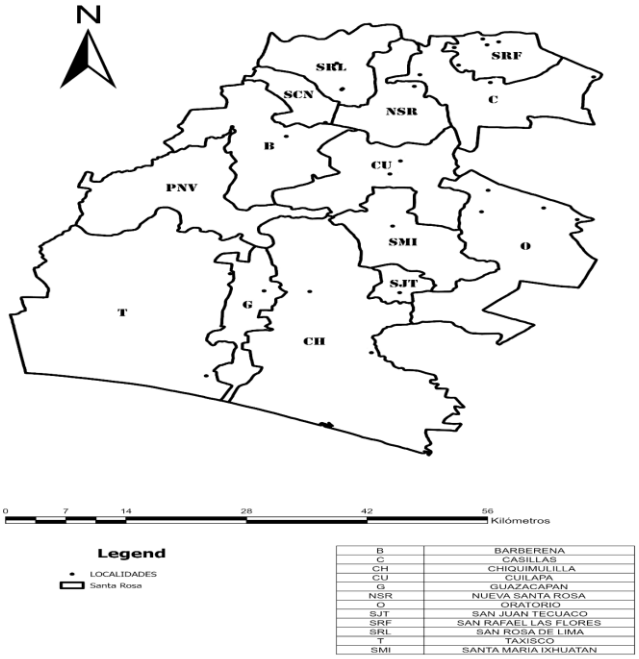

Figure 2. Location of *Ae. albopictus*, positive municipalities and localities in the Department of Santa Rosa during 2024.

46.27% of the samples collected come from the Northern municipalities of Santa Rosa de Lima, San Rafael de Flores, Casillas, and Nueva Santa Rosa. To the South in Chiquimulilla and San Juan de Tecuaco 10 and 6 samples of *Ae. albopictus* were collected respectively. This represents the second and fifth best Absolute Frequency registered in the study. Furthermore, the municipalities of Guazacapán (Center) and Oratorio (East) stand out in terms of samples collected (**Figure 2**).

On the other hand, in the research 12 samples with larvae are identified of the species *Ae. aegypti, Culex quinquefasciatus* and *Culex coronator* coexisting with *Ae. albopictus.*

Table 2. Distribution of the samples of *Ae. albopictus* collected by municipalities and sector of the Department of Santa Rosa, Guatemala, 2024.

Municipalities	Absolute frequency	Relative Frequency (%)	Sector*	
			Urban (%)	Rural (%)
Santa Rosa de Lima	11	16,42	18	82(2)**
Chiquimulilla	10	14,93	35	65(7)
Casillas	9	13,43	31	69(6)
San Rafael de Flores	8	11,94	29	71(4)
San Juan de Tecuaco	6	8,96	30	70(5)
Guazacapán	5	7,46	65	35(9)
Oratorio	5	7,46	40	60(8)
Barberena	4	5,97	81	19(10)

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Nueva Santa Rosa	3	4,48	37	63[8]
Taxisco	3	4,48	31	69[6]
Cuilapa	2	2,99	95	5[11]
Santa María de Ixhuatán	1	1,49	19	81[3]
Pueblo Nuevo de Viña	0	0,00	15	85[1]
Santa Cruz Naranjo	0	0,00	35	65[7]
Total	**67**	**100,00**		

Source: LED Entomological Samples Book. *From the Department of Surveillance and Control of Sanitary Regulations (DVCRS) of the DDRISS of Santa Rosa, Guatemala. **Place in terms of the proportion of the Rural Sector.

Table 3 described that 31.34% (21/67) of the samples were collected during the dry season and 60.66% (46/67) during the rainy season. In 8 of the 12 municipalities *Ae. albopictus* larvae were collected in both seasons.

Table 3. Samples collected by municipalities in different seasons in the Department of Santa Rosa, Guatemala, 2024.

Municipality	Samples collected during the dry season (Nov-Apr.)	Sampled collected during the rainy season (May- Oct.)	Total
Santa Rosa de Lima	4	7	11
Chiquimulilla	2	8	10
Casillas	1	8	9
San Rafael de Flores	0	8	8
San Juan de Tecuaco	1	5	6
Guazacapán	1	4	5
Oratorio	3	2	5
Barberena	2	2	4
Nueva Santa Rosa	2	1	3
Taxisco	3	0	3
Cuilapa	2	0	2
Santa María de Ixhuatán	0	1	1
Total	**21**	**46**	**67**

Source: LED Entomological Specimen Book.

According to **Figure 3**, the highest percentage of *Ae. albopictus* breeding sites were identified in the peridomiciliary. In parallel, 100% (14/14) and 96.23% (51/53) of the samples were collected in the urban and rural sectors, respectively, came from this area of the house.

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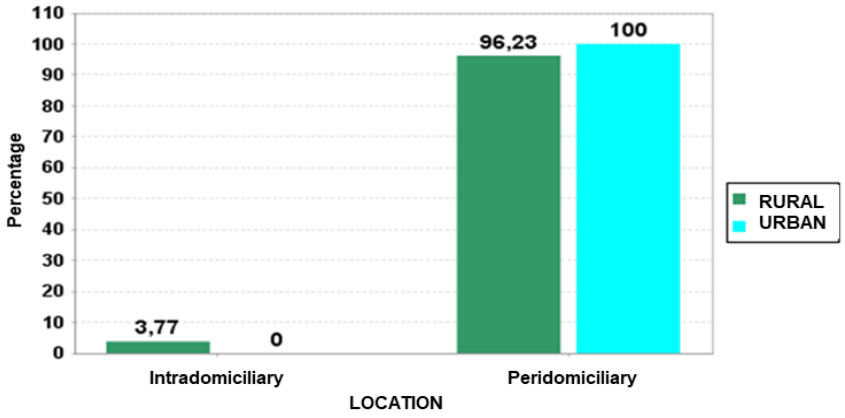

Figure 3. Proportion of the samples collected according to the location of the deposits in the dwelling in the rural and urban sector.Source: LED Entomological Samples Books

In **Table 4** appear the 10 positive deposits to the species *Ae. albopictus* reported during the study. DANU predominated with 61.19% (41/67), among them: tires with 34.33% (23/67) and cans with 16.42% (11/67). This was followed by DAU and DN with 31.34% (21/67) and 7.46% (5/67), respectively. The coexistence of *Ae. aegypti* and *Ae. albopictus* was reported in 7 tires and 2 barrels. No statistically significant differences (p=0.14) were detected between the *Ae. albopictus* positive deposits described in the rainy and dry season.

Table 4. Deposits testing positive to *Ae. albopictus* in the different rainy and dry season in the Department of Santa Rosa, Guatemala, 2024.

Deposits	Rainy period (Nov- Apr.)	Dry season (May- Oct.)	Type of deposit
Tires	14	9	23^{DANU}
Barrels	9	3	12 ^{DAU}
Cans	5	6	11 ^{DANU}
Bottles	7	0	7 ^{DANU}
Pila	4	0	4^{DAU}
Coconut shell	3	1	4^{DN}
Basin	3	0	3^{DAU}
Three hole	1	0	1^{DN}
Vase	0	1	1^{DAU}
Tank	0	1	1^{DAU}
Total	**46**	**21**	**67**

Source: LED Entomological Samples Books.

4. Discussion

The monitoring of arbovirus transmitting mosquitoes are necessary strategies for the control and prevention of possible epidemic outbreaks that affect Public Health [3]. Among the essential aspects for the achievement of that purpose is the knowledge of the spatial distribution and the vector's breeding sites [20].

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Lepe et al. [15] reported in 2017 for the first time the *Ae. albopictus* for the Department of Santa Rosa [15]. In this way, vector capture points are described in the center and north of the Department of Jutiapa, with which Santa Rosa shares a long border that allows extensive traffic of vehicles and merchandise.

In the current study, the annual average values of temperature, precipitation and altitude for the municipalities justify the presence of *Ae. albopictus*. In that sense, it can be mentioned that Lepe et al. [15] in the research about the distribution of *Ae. abopictus* in Guatemala, show the tolerance range of the mentioned species in terms of the values of the climate variables, similar result to the described in the actual investigation.

The low amount or absence of the collected samples of *Ae. albopictus* during the season in the municipality could be associated with the combination of various factors. The Operational Manual for Entomological Surveillance and Control of Guatemala establishes only actions for *Ae. aegypti* [19]. This is not completely effective for the surveillance of *Ae. albopictus,* due to it can coexist along with *Ae. aegypti* [20], and it is generally found away from the houses and frequently in rural areas. Other elements worth highlighting is the implementation of surveys only twice a year and it is indicated just the collection of fourth-instar larvae, mainly in the breeding sites for *Ae. aegypti* [17]. These aspects demonstrate the possible breaches and weaknesses that can appear for the control of *Ae. albopictus*.

The most detailed investigations about *Ae. albopictus* in Guatemala was done by Ogata et al. [14] and Monroy et al. [22]. These authors have been based in the search of the species in situ. Nevertheless, Lepe et al. [15], similar to what was done in the current study, bases the study on the work of operators, which are moderated by the MOVCVED. In both studies, territories with similar conditions appear, to which the species is reported without registration. Hence, the importance of designing surveillance strategies aimed at detecting the mosquito.

Between 1995 - 2025, only three studies referred to *Ae. albopictus* have been published in Guatemala (two about the Department of Izabal and one of all Guatemala) [14,15,22]. This result shows irrefutably the need to promote more studies that expand the knowledge of the species in the country.

On the other hand, between 2023 and 2024, the number of dengue cases was exceeded in comparison to the epidemic years. In the period January-May 2024, there was an incidence rate of dengue cases in the Department equal to 353.2 per 100,000 inhabitants, which placed it in third place in the country [23]. Dengue was the only disease caused by arbovirus during 2024 in the 14 municipalities of the Department of Santa Rosa. 5,096 cases were reported with 14 deaths.

As a result, to control *Ae. aegypti*, from 2023 onwards, intensive dechatting (elimination of disposable containers from backyards), abatization of [15] deposits and fogging were carried out in most of urban and peri-urban areas of the Department. This, probably, impacted the reduction of *Ae. albopictus* breeding sites, since this species can be found sharing the same habitats as *Ae. aegypti.* This is corroborated in the present investigation and other studies carried out in different parts of the world [14,24-26].

Pérez et al. [24] suggest the decrease of the number of larvae of *Ae. albopictus* at the end of 2012 compared to 2011 in various municipalities of the Havana could be due to a rise in the frequency of the chemical adulticide treatments against *Ae. aegypti*.

Juliano et al. [25] concluded in their study that the eggs of *Ae. albopictus*, when subjected to desiccation for 30 days have a high mortality rate. Hence, when the rainy season arrives, there is a notable reduction in the abundance of larvae of the species. According to the average precipitation value for each municipality, drought predominated in 2024 in the Department of Santa Rosa, which could have influenced the low collection of *Ae. albopictus* larvae. The present study also revealed that breeding sites located in the peridomiciles predominated for the species *Ae. albopictus*, which coincides with the results obtained in Cuba, Colombia, Dominican Republic, Congo, and Cameroon studies [26-31].

It is important to mention that the majority of samples of *Ae. albopictus* were found in the rural sector, which is consistent with other studies [32-35]. The municipalities where samples were collected, there is a wide rural portion of the territory. According to Rey et al. [10] *Ae. albopictus*

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predominates in rural areas because females need to feed on nectar and prefer to lay eggs, rest, and bite outdoors. In addition, this species is opportunistic in the selection of blood food sources, in contrast *Ae. aegypti* prefers fundamentally blood of human origin.

On the other hand, Monroy et al. [22] show in the Department of Izabal, Guatemala, that *Ae. albopictus* habitat mainly in the urban sector, the species was found in 55.6%. In the same way, other authors show the presence of this species in the urban environment [29,30,36]. One possible explanation is the fact that in these areas there are sectors with abundant vegetation covered with favorable conditions for the habitat of this species. This was evidenced by the collection of *Ae. albopictus* larvae in the parks and green areas of Ibagué, capital of the Department of Tolima, Colombia [3]. Evidently, this fact increases the possibilities of *Ae. albopictus* contact with humans, since large numbers of population are concentrated in urban areas and, given the presence of another vector (*Ae. aegypti*). The situation could complicate the transmission and control of arbovirus.

In the current study, the diversity of breeding sites for *Ae. albopictus* with predominance of DANU was demonstrated, in particular tires and cans, similar results were obtained in other studies developed in Guatemala [14,22], Cuba [24] and Cameroon [31]. The predominance of positive DANU of *Ae. albopictus* in the peridomiciliary suggests that is that area where families have less action with respect to sanitation. Similar results were obtained by Rodríguez et al. [30] in one research carried out in the Dominican Republic. In the totality of the analyzed municipalities can be observed average values of low annual precipitation [16], which could have influenced the reduction of the breeding sites for *Ae. albopictus.*

In relation to tires, it constitutes one of the fundamental tracks for the dispersion of immature phases of the species [24], especially eggs [3]. Egid et al. [37] identified that, possibly, these sites are less vulnerable to perturbation than other recipients such as cans and coconut shells. Furthermore, internal conditions of reduction of lights and humidity make them especially attractive for the *Aedes* mosquitoes.

In the Department of Santa Rosa there is wide circulation of tires on the inside and outside of the territory, which constitutes an important risk factor for the propagation of the mosquito. It is stated the preference of *Ae. albopictus* larvae in the breeding sites where there is water turbidity that indicates the presence of organic materials that can supply food resources and serve to hide aquatic stages from predators [30]. This justifies the majority of these breeding sites in the current study have been highlighted by the found deposits coexisting with *Ae. aegypti.*

The fact that no significant statistical difference was found between the positive deposits of *Ae. albopictus* detected during the rainy and dry season indicate the importance of establishing rules in the program of surveillance and control vector for the species that are sustainable over time. In this context, it is important to mention that the dry season constitutes a crucial moment for preventive actions, because the *Ae. albopictus* eggs have the characteristic of being resistant to adverse conditions and hatch when these improve, as it happened during the rainy season [34].

Among the limitations of the study are the analyzed sample of *Ae. albopictus* is small and variable. Likewise, other variables such as light, food, air strength, and more should be studied to provide a holistics picture of this phenomenon. However, this study shows interesting results that need to be analyzed for the control of *Aedes albopictus* in the Department of Santa Rosa.

5. Conclusions

In conclusion, the presence of *Ae. albopictus* was found in the majority of the municipalities of the Department. At the same time, it is corroborated by other authors about the ecological plasticity of this species [1,3,6,9], which allows the propagation in rural and urban areas, occupying a broad range of breeding sites, especially the DANU, many which have been guaranteed as a result of human activity. Finally, the coexistence of *Ae. albopictus* with other species of medical importance is described, opening the doors to new lines of research.

Detailed studies of *Ae. albopictus* are necessary in neighborhoods, colonies, hamlets, and farms that provide information about the bionomy of the vector, its relation with viruses and implication in the transmission of diseases. In the same sense, it is of great importance the establishment of

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surveillance and control measures for the "Asian tiger" population through the Vector-Borne Diseases Program of Guatemala.

Supplementary Materials: No supporting information was downloaded.

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Abbreviations

The following abbreviations are used in this manuscript:

DENV	Dengue virus
CHIKV	Chikungunya virus
ZIKV	Zika virus
YEV	Yellow fever virus
MOVCVEG	Operational Manual on Vector Surveillance and Control for Guatemala
LED	Departmental Entomology Laboratory
MSPAS	Ministry of Public Health and Social Assistance of Guatemala
UAD	Useful Artificial Deposits
NUAD	Non-Useful Artificial Deposits
ND	Natural Deposits
DDRISS	Departmental Directorate of Integrated Health Services Networks of the Department of Santa Rosa.
DVCRS	Department of Surveillance and Control of Sanitary Regulations

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