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A survey of tire-breeding mosquitoes (Diptera: Culicidae) in the Dominican Republic: considerations about a pressing issue

Tire-breeding mosquitoes in the Dominican Republic

Estudio de mosquitos (Diptera: Culicidae) que se desarrollan en neumáticos en República Dominicana: consideraciones de un problema acuciante

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Introduction: Discarded vehicle tires represent a serious threat both to the environment and to the public health as stored tires have the potential to harbor important mosquito (Diptera: Culicidae) vectors.

Objective: To assess the importance of used vehicle tires as larval habitats for mosquito fauna that colonize these artificial reservoirs in Jarabacoa, Dominican Republic.

Materials and methods: Used tires were sampled with pipettes in specialized tire fitting shops and scattered stockpiled tires between June and August 2018.

Results: 396 tires were sampled; 57 (Container index, CI = 14.4 %) were positive to immature stages, containing 2,400 specimens, 11 species and four genera (*Anopheles, Aedes, Culex* and *Toxorhynchites*). The most abundant species was *Aedes albopictus* (42.3 %), followed by *Aedes aegypti* (34.3 %), and *Culex quinquefasciatus* (14.0 %), among others (9.4 %) less abundant. The CI varied significantly among the different tire sizes (X^2= 13.4; $P \leq 0.05$). The highest infestation levels were found in the largest size tires. A low positive correlation (r = 0.38, n =396; $P \leq 0.001$) between the tire size class and immature stages prevalence was recorded. The presence of organic matter had an overall positive effect on the infestation levels (U = 11430.0; $P \leq 0.001$).

Conclusions: These rubber-residues usually located nearby human populations represent important breeding sites for vectors of arboviruses such as dengue, chikungunya, Zika or West Nile.

Key words: Culicidae; *Aedes*; arboviruses; tires; Dominican Republic.

Introducción. Los neumáticos usados de vehículos representan una grave amenaza tanto para el medio ambiente como para la salud pública, ya que actúan como lugares de cría de especies de mosquitos (Diptera: Culicidae) vectores de enfermedades.

Objetivo. Evaluar la importancia de neumáticos usados como hábitats larvarios de mosquitos que colonizan estos recipientes artificiales en Jarabacoa, República Dominicana.

Materiales y métodos. Neumáticos usados fueron muestreados mediante pipetas en lugares especializados como tiendas de venta, reparación y vertederos, entre los meses de junio y agosto de 2018.

Resultados. Se muestrearon 396 neumáticos; 57 (Índice Contenedor, IC = 14.4 %) albergaron estados inmaduros, conteniendo 2.400 especímenes de 11 especies y cuatro géneros (*Anopheles, Aedes, Culex* y *Toxorhynchites*), La especie más abundante fue *Aedes albopictus* (42.3 %), seguido de *Aedes aegypti* (34.3 %) y *Culex quinquefasciatus* (14.0 %), entre otras (9.4 %) de menor abundancia. El IC varió significativamente con el tamaño de neumático (X^2 = 13.4; $P \leq 0.05$), mostrando los mayores niveles de infestación en los neumáticos de mayor tamaño. Una baja correlación positiva (r = 0.38, n = 396, $P \leq 0.001$) fue observada entre el tamaño de neumático y la prevalencia de estadios inmaduros. La presencia de materia orgánica tuvo un efecto positivo sobre los niveles de infestación de mosquitos (U = 11430.0; $P \leq 0.001$).

Conclusión. Estos residuos de caucho, generalmente almacenados cerca de poblaciones humanas, representan lugares propicios para la reproducción de vectores de arbovirus tales como el dengue, chikungunya, Zika o West Nile.

Palabras clave: Culicidae; *Aedes*; arbovirus; llantas; República Dominicana.

Management of waste is a demanding and challenging process in developed countries, with important implications for human health, well-being, environmental preservation, sustainability, and economy (1). Among solid waste, waste or scrap tires (defined as used, discarded or rejected tires that are either whole or in pieces) represent a serious environmental concern on several fronts: toxins released from tire decomposition, incineration or accidental fires can pollute the water, air and soil (2); also abandoned or outdoor stored tires can harbour disease vectors, particularly rodents and mosquitoes (Diptera: Culicidae).

With regard to the public health role of used tires, its trade has been demonstrated as a mechanism for the worldwide dispersal of container-breeding mosquitoes in past decades (3). This long-distance spread is explained because almost all aedine species lay desiccation-resistant eggs that can persist and survive for long periods away from free water (4). When the water level inside tires rises due to rain, the eggs can hatch and facilitate mosquito invasion into new areas (5). Discarded tires provide ideal breeding sites as the tires design holds water and offers shade (reduced light) whereas the rubber creates a suitable environment (safe place) for hatching eggs (6). Service stations, tire dealers, tire repair storage shops and salvage yards are common sources of discarded tires (7). Many tire dealers and tire fitting shops usually lack appropriate storage sites, hence they accumulate large quantities of used tires in their facilities, plots and surrounding areas frequently located outdoors and near to human habitation (8). Many mosquito species that inhabit tree holes find these man-made containers a supplement to their natural oviposition sites (3), therefore these tires have the potential to affect the

epidemiology of mosquito-borne diseases and become a public health hazard (9,10).

Mosquito-borne diseases represent a serious public health problem in the Americas and especially in the Caribbean which are becoming an obstacle to the economic development. In the Dominican Republic, some recent studies have dealt with mosquito breeding hotspots. Borge de Prada *et al*. (11) and Rodríguez Sosa *et al*. (12) showed that the accumulation of waste in public spaces allows the proliferation of mosquitoes of public health importance, whereas Rodríguez Sosa *et al*. (13) observed this problem also in the domestic environment. On the other hand, González *et al*. (14) showed the relevance of artificial container as important urban foci with high density of vector mosquito species in urban cemeteries.

However, these studies are still very limited in the Caribbean region. Hence, the objective of this research was to assess the importance of used vehicle tires as larval habitats for mosquito fauna by assessing the species composition, relative abundance and correlate mosquito numbers with some attributes such as size tire class, Container Index (CI) and presence/absence of decaying detritus in periurban areas of the Dominican Republic.

Materials and methods

Study area

The study was performed in the municipality of Jarabacoa (La Vega Province, Dominican Republic), which has a population of ca. 32,600 inhabitants within an area of ca. 660 km^2. Jarabacoa has a typical tropical rainforest climate with 1,340 mm of annual rainfall, 22.9 ºC of annual daily mean temperature and

Köppen climate classification: Af. The city is situated in a strategic position to develop mountain tourism and ecotourism.

Data collection

In this study, an attempt was made to locate the majority of sources and accumulation of used vehicle tires. Discarded tires were surveyed from June to August 2018 in four main tire repair storage shops (known as «gomeras» by locals) with tire piles accumulated outdoors, and also other two small abandoned stockpiles (containing 2 and 3 tires, respectively) located in public spaces (figure 1A-B). Informed consent was obtained for each of the sampled private properties. All water-filled tires (positive or negative) were counted and classified according to the tire size in four classes: motorcycle, car, truck, and tractor tires, with the mean tire rolling diameter of 64, 76, 102, and 178 cm, respectively. The presence of organic matter was recorded as positive (input of decaying leaves, plants or fine detritus) or negative (no organic matter or just a thin layer of it) in water-filled tires. If present, all mosquito larvae and/or pupae were collected from each tire to plastic trays, and then the content was transferred to hermetic tubes using disposable pipettes. No tire was sampled more than once.

In the laboratory, immature stages of mosquitoes were transferred alive into mosquito breeders (Bioquip Products, USA) with their own water (figure 1C). Collected larvae were killed by placing them in hot water (60 °C) for 1 min and after that, fixed in 70 % ethanol once they reached the IV instar. Pupae were raised to adults and then killed by freezing to ensure an accurate identification. Both larvae and adults were identified using the key of González Broche (15). Voucher specimens are deposited in the Laboratory of Entomology of

Universidad Agroforestal Fernando Arturo de Meriño (Jarabacoa, Dominican Republic).

Statistical analysis

Data analysis was carried out to evaluate the influence of tire size class on the CI and immature stages prevalence. We run the analysis using non-parametric tests after assessing data were not normally distributed. CI was calculated as the number of positive tires/number of sampled tires * 100. CI was compared between the four classes of tires by Chi-Squared (X^2) test followed by pairwise comparisons. Spearman correlation coefficient assessed the association between tire size classes and mosquito prevalence. Along the manuscript, data are presented as medians with corresponding ranges in brackets. The input of organic matter inside water-filled tires on the mosquito immature was assessed by U-Mann-Whitney test. Descriptive analysis and statistics were conducted using IBM SPSS V22.O software (alpha-level = 0.05).

Results

Out of 396 used vehicle tires sampled, 227 (57.4 %) were water-filled tires and 57 (CI = 14.4 %) harbored immature stages of mosquitoes. In total, 2,400 immatures (2,279 larvae and 121 pupae) of 11 mosquito species and four genera (*Anopheles, Aedes, Culex* and *Toxorhynchites*) were collected and subsequently identified (table 1). *Aedes (Stegomyia) albopictus* (Skuse) accounted for most of the collections (1,017 specimens, 42.3 %), followed by *Aedes (Stegomyia) aegypti* (Linnaeus) (825 specimens, 34.3 %), and to a lesser extent, *Culex (Culex) quinquefasciatus* Say (336 specimens, 14.0 %). The remaining 9.4 % belonged to other eight mosquito species heterogeneously distributed (table 1). Tires containing immature mosquitoes had a median of 24

(216) specimens/tire (table 2). The water content inside tires varied from 49 % in cars to 90 % in motorcycles. Most of the used vehicle tires belonged to the class of cars (80.1 %), followed by truck tires (14.1 %), and to a minor extent the remaining classes (≤ 6 %). The lowest species richness was found in motorcycle tires with no Culicidae collections and the highest was found in car tires containing all species collected (table 1).

CI significantly varied among the different tire size classes (X^2 = 13.4; gl = 3; *P* = 0.04) with significantly higher prevalence rates in truck (X^2 = 18.2; P = 0.032) and tractor tires (X^2 = 11.8; P = 0.028) than in car tires. The highest CI was recorded in truck tires (CI = 40 %) in contrast to the smaller tires of motorcycles (CI = 0 %) (table 2). Although recorded in lower numbers, *A. aegypti* accounted for a higher total CI than its congener *A. albopictus* (table 2).

A low positive correlation (r = 0.38, *P* ≤ 0.001) between the tire size class and prevalence of immature stages was recorded. Overall, the immature mosquito prevalence rates varied significantly according to the vehicle tire size class (X^2 = 18.2; *P* ≤ 0.001). A higher number of specimens in tractor tires: 119 (145) was observed compared to car tires: 16 (216), truck: 39.5 (163), and motorcycle tires: 0 (0) (table 1).

Inhabited tires with organic matter was recorded in 42 (74 %) from the total sampled tires. The presence of organic matter (with organic matter = 26 (216); without organic matter = 16 (105) inside discarded tires had a positive effect on the prevalence of immature stages of mosquitoes (U = 11430.0, *P* ≤ 0.001). The same tendency was also observed in the other three most common species collected.

Discussion

To our knowledge, this survey represents the most comprehensive study published in the region about used vehicle tires and draws attention to some important aspects discussed here. In recent times, there has been an increasing interest in the study of vectors whose breeding sites are located in environments that accumulate rubbish and waste (16). In Central America, studies on mosquito fauna in discarded tires are relatively common (17-20). All these studies showed the relevance of tire-breeding habitats as an important locus for mosquito species of public health significance. In the Dominican Republic, e.g. used tires have been also reported to be, to a greater or lesser degree, one of the key containers (11-13,21-24).

Our survey revealed important aspects, e.g. (i) tires support high species richness comprising nearly 70 % of the known species spotted in the studied district, (ii) moderate infestation levels of *A. aegypti* and *A. albopictus* immature stages were recorded on these particular breeding habitats in agreement with literature published elsewhere (25,26), (iii) mosquito CIs varied significantly across the four tire size classes; lower levels of infestation were found in smaller tires (motorcycles) in contrast to larger tires (truck and tractor) as reported by McMahon *et al*. (9), which, in turn, noted that bigger tires had more surface and stored water volumes for up to 200 l (7), being therefore more prone to serve as breeding sites for mosquitoes. The overall CI obtained in our study was similar to those reported in other regions, e.g. Argentina (total mosquitoes, CI = 17.7) and (CI = 11.6) for *A. aegypti* (27), and (iv), the input of organic matter in discarded tires had a positive effect on numbers of immature mosquitoes in line with other studies (28,29), thereby corroborating the

contribution of the organic matter (detritus, leaves, twigs, seeds, etc.) to larval nutrition producing larger females and number of specimens.

Aedes aegypti and *A. albopictus* are two aedine mosquito species of cosmopolitan distribution, which prefer laying their eggs in artificial containers like discarded tires. In fact, they are called «tire-breeding mosquitoes» since in many cases these containers become the most preferred breeding habitats (30,31). In the island of Hispaniola, e.g. water storage tanks were the most frequent positive container for *A. aegypti* followed by used car tires (32). In the Dominican Republic, discarded tires are important for the proliferation not only *Aedes* spp. but also for *Culex* spp., both in the domestic and public environments (11,13). In this regard, *Culex*, mainly *C. quinquefasciatus*, is frequently reported as a common tire-breeding mosquito along different countries of the Caribbean region and Latin America (27,33). In addition to *Aedes*, some species of anopheline mosquitoes are also dispersed through human activities, e.g. movement of used vehicle tires (34). We found *Anopheles (Anopheles) grabhamii* Theobald and *Anopheles (Anopheles) crucians* Weidemann breeding in a single car tire. In the case of the latter species, this represents the first record for Jarabacoa according to the checklist of Rodríguez Sosa *et al*. (12). *Anopheles (Nyssorrhynchus) albimanus* Weidemann, known as the main vector of malaria in Central America including the Caribbean territory, was previously reported breeding in used tires in the municipality (11), but not in the present study. These findings revealed that these anopheline species that generally are found in different types of natural ecosystems (14,35) may also breed in artificial containers at low frequency, which might contribute to be found in urban areas as reported by Mendizábal Alcalá *et al*.

(36). On the other hand, the presence of *Toxorhynchites (Lynchiella) portoricensis* (von Röder) was occasional, although it seems to breed in tires more frequently than Dominican *Anopheles* spp., as noted in the literature (12,15).

Waste management regulations are country-specific and should be implemented to promote the environmentally sound management of waste tires by providing a regulatory framework. According to the Resolution NO.005-2015 stated by Ministry of Environment and Natural Resources of the Dominican Republic, it is prohibited to deliver or discard used tires in unauthorised dumps, streams and water courses, as well as in other places such as parcels, plots, wasteland, and backyards to avoid the accumulation of water and propagation of vectors (37). However, despite the above, it is common that discarded tires are improperly stockpiled or illegally dumped in the country. This situation is worrying especially in service stations, tire dealers, auto repair shops, tire fitting shops, and salvage yards, many of them lacking a registered waste tire storage sites. These business have large accumulations of waste tires stored outside their facilities, sometimes even on the roof and in surrounding areas as can be observed along the Duarte Highway on the way to Santo Domingo (capital city of the Dominican Republic).

Taking into consideration the almost 7 billion people in the world, 1,1 billion vehicles on the road, 1,7 billion new tires produced a year and up to 1 billion waste tires generated per year, recycling and reuse of rubber should be a human obligation. Recycling of waste tires has been widely studied over recent decades in applications related to infrastructure and civil construction, especially asphalts, concretes and isolators for lightweight construction,

waterproofing systems, and membrane liners, among others (38,39). Nowadays, inexpensive and eco-friendly hand-made alternatives are becoming more common, e.g. reconvert scrap tires into furniture, decoration, toys, flowerpots, etc. which are also an effective way for community renewal initiatives to promote artisanal handicraft.

In conclusion, among artificial containers, discarded vehicle tires represent an important source of mosquito vectors in many countries, and also in the Dominican Republic. Despite international and national guidelines warning about the properly dispose and storage of vehicle tires, it seems clear that this urban solid waste is not being considered a serious threat in mosquito control activities in the country, as seen in other places (27). Therefore, it is imperative the implementation of the legal procedures to manage this waste both at municipal and national level in line with other countries (40). It is also recommended to strengthen preventive measures with awareness campaigns aimed at informing, sensitizing and mobilizing communities to eliminate the risks associated with the presence of mosquito-borne diseases as has recently been carried out in the municipality of Jarabacoa by Vásquez Bautista *et al*. (41). It is also essential to undertake a more thorough cleaning of wastelands in order to reduce the health risks related to the presence of mosquitoes. Ultimately, it is strongly recommended the practice of waste reduction, reuse and recycling.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Figure 1. Sources of discarded vehicle tires and mosquito breeders in the laboratory: A) car used tires stacked in a service station against a fence, B) mixed vehicle tires abandoned in a fitting shop, and C) mosquito breeders for rearing pupae into adult's stage.

Table 1. Summary of information related to the immature mosquitoes collected in vehicle tires from Jarabacoa, Dominican Republic (June-August 2018).

Attributes	Tire size class				
	Motorcycle	**Car**	**Truck**	**Tractor**	**Total**
Tire rolling diameter (Ø, cm)	64	76	102	178	-
Sampled tires (n)	11	319	56	10	396
Positive tires (n)	0	39	14	4	57
Container index (%)	0	12	25	40	14
Water-filled tires (n)	9	158	54	6	227
Organic matter in water-filled tires(%)	0	29	9	4	42
Specimens (n)	0	1,179	790	431	2,400
Median specimens/tire (range)	0 (0)	16.0 (216)	39.5 (163)	119.0 (145)	24.0 (216)
Species richness	0	11	2	4	11

Table 2. Mosquito species identified in different types of discarded tires of Jarabacoa, Dominican Republic (June-August 2018).

Species	Tire size class *					
	Motorcycle no. (%)	**Car no. (%)**	**Truck no. (%)**	**Tractor no. (%)**	**Total no. (%)**	**CI (%) ***
C. quinquefasciatus	0	240 (20.3)	62 (7.8)	34 (7.9)	**336 (14.0)**	**4.3**
C. corniger	0	7 (0.6)	0	0	**7 (0.3)**	**0.2**
C. atratus	0	14 (1.2)	0	0	**14 (0.6)**	**0.2**
C. secutor	0	6 (0.5)	0	0	**6 (0.2)**	**0.5**
C. nigripalpus	0	2 (0.2)	0	71 (16.5)	**73 (3.0)**	**0.7**
T. portoricensis	0	70 5.9)	0	0	**70 (3.0)**	**0.2**
A. albonotatus	0	47 (4.0)	0	0	**47 (1.9)**	**1.3**
A. aegypti	0	313 (26.5)	300 (38.0)	212 (49.2)	**825 (34.3)**	**11.6**
A. albopictus	0	467 (39.6)	436 (55.2)	114 (26.4)	**1,017 (42.3)**	**7.6**
An. grabhamii	0	1 (0.1)	0	0	**1 (0.1)**	**0.2**
An. crucians	0	4 (0.3)	0	0	**4 (0.2)**	**0.2**
Total	**0**	**1,179**	**790**	**431**	**2,400 (100)**	**14.4**

* Container Index.