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Mosquito Diversity and Public Services as Risk Factors for Emerging Diseases in a Small Village, Ecuador Amazon

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ABSTRACT
The creation and/or extension of urbanisms towards jungle areas, deficiencies in public services, tourism, hunting practices and exploitation of natural resources have been important factors that influenced the epidemic outbreaks of arboviruses in the Amazon. Limoncocha is a small population of the Kichwa ethnic group created in the 1950's by the Linguistic Summer Institute, with a current population of about 1,500 inhabitants, immersed in the Biological Reserve of Limoncocha, RAMSAR (1998). A systematic, stratified and random sampling of 80% of the total inhabited houses (65 dwellings) was carried out, using entomological sampling and surveys on socio-environmental factors. A direct relationship between the deficiency of public services and the breeding of urban and jungle vectors was found, this allowed estimating the vulnerability and risk factors present for the local transmission of arboviruses in the community. The analysis suggested that the population had the permanent water for consumption through different routes, but only 8% of homes had the potable water pipe system, and half of these households had daily interruptions in their supply. 90% stored water collected from rivers and streams, in useful containers of low volumes, such as buckets and face-washers, from tankers, cisterns, and wells. A third of the homes (25%) did not have a waste collection service, 40% of the ones with the service, did not remove the inorganic ones, which represented 60% of the positive recipients for mosquitoes. The index of the positive containers per household was low (0.5, max = 4, min = 0). The breeding pattern was determined by the waste containers with mainly sylvan species. The low aedic index (1.5%), suggested a low colonization by Aedes aegypti in the locality, and that the cases of Dengue/Chikungunya reported were not of the local transmission, having the greatest vulnerability, and the possible translocation of sylvatic pathogens from the biological reserves by sylvatic vectors through the ecotone-periphery-center of the village gradient.

Keywords: Arbovirus, *Aedes aegypti*, mosquitoes, potable water, inorganic waste, disease ecology.

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INTRODUCTION

The creation and/or extension of urbanisms towards jungle areas, deficiencies in public services, tourism, hunting practices and exploitation of natural resources have been important factors that influence the epidemic outbreaks of arboviruses in the Amazon [1-4]. The increase of vectors carrying emerging pathogens, such as *Aedes aegypti* and other (urban species in Latin America, has been associated with the urbanization without prior planning in land use planning and/or deficiencies in public services. This association has been given by continuous and prolonged interruptions in the supply of drinking water; the absence of services for the final disposal of inorganic waste; and, ultimately, to social customs [5-7] These factors have promoted the accumulation of the containers of anthropogenic origin, whether utilitarian, waste and ornamental, which have acted as the artificial

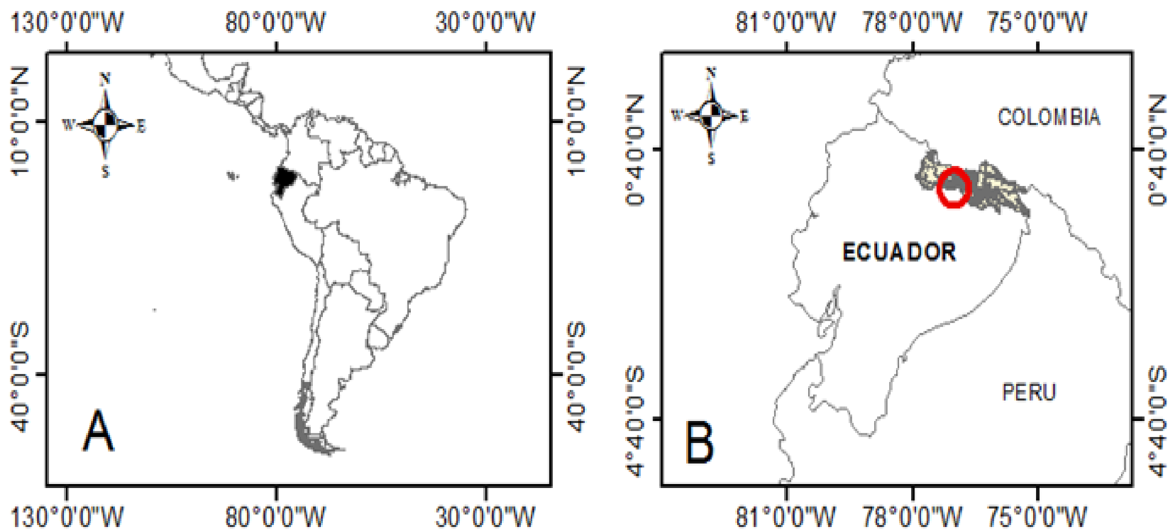
breeding sites for immature stages of mosquitoes. However, the ecological changes in natural and/or jungle areas have been the cause of the occasional outbreaks of viruses that remain in sylvatic cycles; the Mayaro virus (MAYV) and Chikungunya (CHIKV) have been the clear example of this situation [8-13].
The development of the diseases related to the unplanned growth of the population centers, the deficiency of services and/or ecological changes has been evidenced in the rural town of Limoncocha, which due to its origin (founded by the Summer Institute of Linguistics and oil companies in the 1950s), geographical location (Amazon), social customs associated with the resident Kichwa community, as well as its immersion in a protected area (Biological Reserve of Limoncocha, RAMSAR 1998), has raised interesting questions regarding the dynamics of vectors and their relationships with socio-environmental variables.
In the population of Limoncocha, there have been several cases of respiratory, intestinal and vector diseases. Among the latter, the most common has been dengue, but the recent cases of Chikungunya and other diseases (Malaria, Leishmaniasis) have been reported [14]. However, there has been no information on the locality regarding the entomological and socio-environmental factors that propitiate the establishment of an endemic and / or epidemic cycle. In parallel, the fact of being immersed in the Biological Reserve should also assess the vulnerability and potential risk to pathogens related to the RBL and their translocation to the urban human population in the continuous contact with the reserve.

This study evaluated the application of an intensive and rapid method [15], the quality of the public services and the diversity of mosquitoes, in order to establish the risk of the population of Limoncocha to the transmission of diseases through vectors/urban and jungle pathogens associated with the town of Limoncocha and/or the Biological Reserve of Limoncocha. The results have allowed determining the vulnerability and risk and establishing the vector prevention strategies. Socio-environmental factors such as deficiency in drinking water services and inorganic waste disposal would be determinant variables for the breeding and establishment of the populations of the urban and jungle mosquitoes in the urbanized areas that increase the vulnerability and risk of the Kichwa population, a hypothesis that was tested in this study.

2. METHODS

Study area

The population of Limoncocha is located in the Limoncocha Biological Reserve (RBL) in the Ecuadorian Amazon, Napo Biogeographic Province, and placed in the political province of Sucumbíos, Canton Shushufindi (00 ° 24'25 ''S; 76 ° 37' 14''W), parish of Limoncocha, at 203 m of altitude and with a total area of 59 853.32 ha [14]. Its geographical limits are to the north with the parishes of Shushufindi and San Roque, to the south with the province of Orellana, to the east with the parish of Pañacocha and the province of Orellana and to the west with the Province of Orellana (Figure 1).



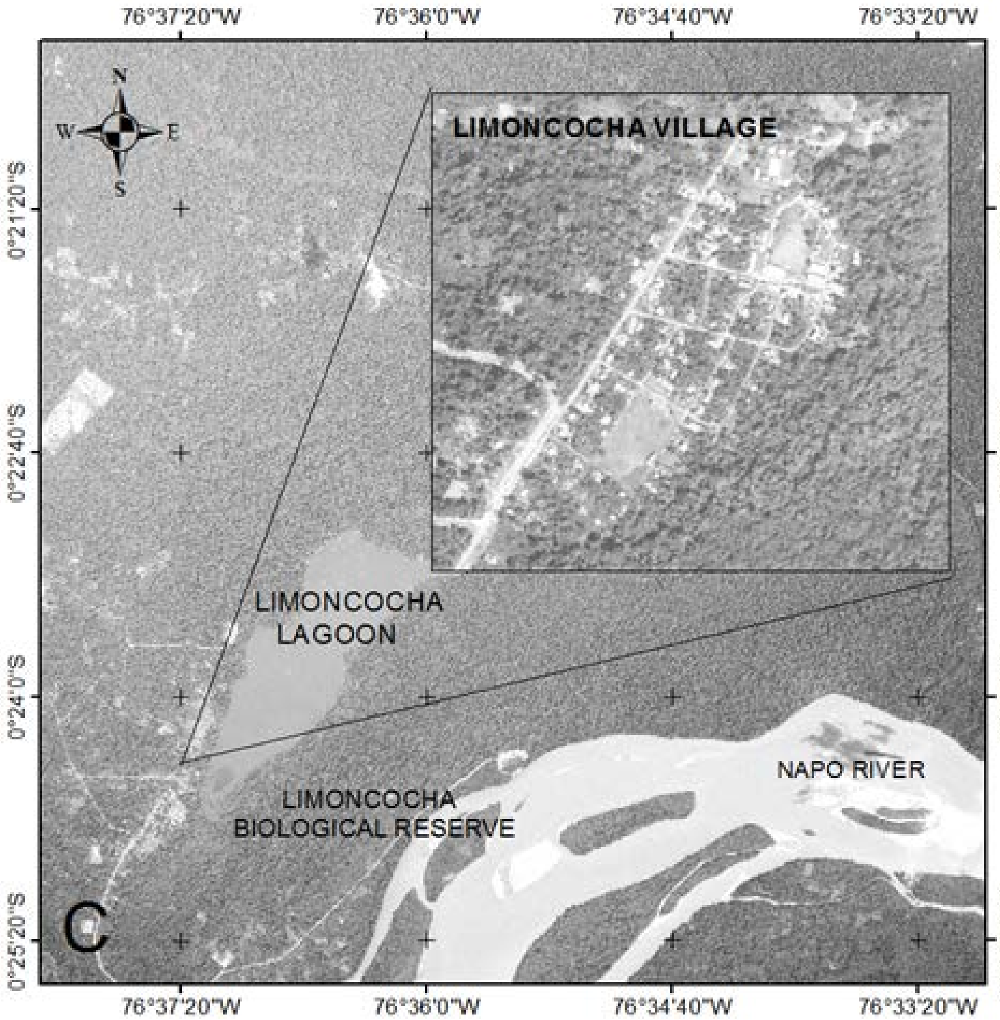


Figure 1. Study area. Population of Limoncocha, Sucumbios Province, Ecuador.

The RBL is limited to the north with the Blanco River, to the east with the Itaya River, to the south with the Orellana Province (Indillama River), to the Northwest with the Yasuní National Park, and to the West with the Jivino River and some populated centers like Playayacu, San Antonio, Limoncocha and Pompella (from north to south). The average annual temperature is 24.9 ° C, while the average annual rainfall is of 2 965 mm [16].

The total population of the parish of Limoncocha consists of approximately 6 700 residents, while in the town, there are approximately 1 500 residents of the Kichwa ethnic group, and education, oil and agricultural activities are the population's main activities [17]. Other declared activities are fishing and hunting in the RBL. The population consists of approximately 23 housing blocks and 120 buildings. Adjacent to the town is the Napo River (1-1.5 km away) and the Limoncocha Lagoon (abandoned meander of the Napo River, 1,230 years). The RBL is a RAMSAR area (wetland, declared in 1998), being one of the smallest protected areas in Ecuador (4 613 ha.), and it was declared as a Natural Reserve on September 23, 1985 [18].

Sample design: stratification and sampling in housing

Based on a map of the urban area product of the satellite image digitization (Landsat), areas or neighborhoods were identified and stratified, and, for each block, houses were selected to gather the data and samples in the highest percentage of the possible homes. In situ, the geographic coordinates (GPS) of each corner/block were taken to verify and correct the information of the satellite images.

A total of four teams with the same number of operators carried out the entomological sampling and the collection of the socio-environmental data. A total of 63 homes were taken, representing 80% of the infrastructure observed in the image. The remaining 20% of homes were not collected as a result of not having been located during the sampling days.

In each household that included a code number and was verified on the map, the adult representative who was present at that moment at home was interviewed. The interview was conducted through a socio-environmental survey, in which the key data for the development of this project were consulted, such as: the number of inhabitants in the dwelling, the type of water supply (pipe, tanker, river, well, etc.), the quality of the supply (interruptions, frequency of them), the collection of the organic and solid waste, the occurrence of febrile cases (last week, month, six months, year), and the information related to the diseases diagnosed in the members of the dwelling. The methodology followed the same process as was indicated in [7-15].

The sampling was carried out for three consecutive days (Feb 2017), collecting adults and immature mosquitoes (Diptera: Culicidae) with different capture methods for a rapid assessment that has previously been shown to effectively estimate the population of the vectors and their diversity for eco-epidemiological purposes [15].

The sampling of socio-environmental information (survey)

To estimate the degree of deficiency in the water supply, the following indicators were taken into account: the type of supply, the interruptions in supply, the frequency and duration of the interruptions, and the storage of water for consumption. On the other hand, the indicators of waste collection were: the availability, frequency and removal of the organic waste and out-of-use containers that acted as nurseries (tires, plastic material, buckets, pots).

Febrile cases and disease reports

To assess the occurrence of febrile cases per dwelling, the clinical diagnosis was asked, as well as the antiquity of the following diseases: Dengue, Zika, Chikungunya, undifferentiated fevers, strong bone pains, scabies, Leishmanisis, Malaria and Chagas.

Entomological methods

With the authorization of the head of the households, along with the application of the socio-environmental survey, the information of types of containers with stored water that served as breeding sites (positive to larvae) for mosquitoes was collected. The data was recorded in a form with the code / house number. The samples were collected and stored, and then processed at the scientific station in situ and laboratory in Quito. The described protocols followed what was described in [7,15,19,20].

Adult and immature phases were collected to obtain the richness representative of the Limoncocha vector population. Similar studies showed that when the vector capture was only focused on a particular development phase, there was an underestimation of the species richness; the same happened when the artificial and natural breeding sites were not considered, since their faunal composition was usually different [15]. In this way, the collection of the specimens was carried out by means of the plastic suckers for the larvae and pupae in containers; mouth aspirators and light traps CDC for adults.

Entomological indices

Vector indices and containers as breeding sites

Entomological indices of common vectors were calculated, such as the *Aedes* index (AI), the Breteau index (BI) and the container index (CI). The first refers to the percentage of the positive houses of *Aedes aegypti* in a given place. The BI is the percentage of the positive containers of *Aedes aegypti* in the total number of houses inspected in a locality, while the CI calculates the percentage of positive Culicidae containers in the total number of it in a given place [7].

Richness and abundance of species

The richness and abundance of mosquito species were calculated by neighborhoods and nurseries classified as utilitarian, ornamental and waste. Conglomerate (CA) and principal component (PCA) analyses were performed (to indicate a reduction by axes of the total of variables that explained the total variance of the presence and abundance of the vectors). The neighborhoods mapped significant data and indexes to determine the spatial vulnerability.

3. RESULTS

Socio-environmental data: Public services and diseases

90% (57) of the houses stored water, whose origin was varied: 43% (27) took it from wells and groundwater, 13% (8) obtained it from tankers, 35% (22) got it from the rainwater and streams, and 10% (6) presented piped water service which completed 100% water availability in the population.

The garbage and waste collection service covered 75% of the homes, however, in 40% of the cases, this service did not remove the non-degradable inorganic solid waste.

The storage of water was carried out in different containers of the anthropogenic origin (artificial and utilitarian), the buckets of 5lt (49%) being the most frequent, followed by tanks (23%) and finally by "face washers" and barrels of 200 liters with a 15 % each. These containers have been considered useful, and made up 42% of the recipients that tested positive for mosquitoes, while the waste containers tested positive for mosquitoes by 54%. Ornamental vases or flower containers were positive by 4%.

The local demography resulted in 3.8 adults and 3 average children per dwelling, which meant six to seven inhabitants per dwelling. Epidemiologically, 57% reported having at least one Dengue, Zika or Chikungunya event a year ago (58%), six months ago (31%) and one month ago (11%). Febrile cases occurred in 62% of the households surveyed, a year ago (41%), six months ago (28%), a month ago (21%), last week and during the time the survey was conducted (10%).

Severe bone pain was reported by 65% of the respondents, which occurred a year ago (34%), six months ago (24%), a month ago (20%), last week and at the time the survey was conducted (22%). Episodes of scabies and mountain leprosy (Leishmaniasis) occurred in 19% of the surveyed dwellings, a year ago (25%), six months ago (42%), a month ago (17%), last week and during the survey (16%). 30% responded having had at least one episode of Malaria and Chagas a year ago (79%), six months ago (11%) and 1 month ago (11%).

Entomological indicators.

Vector indices and breeding sites

The *Aedes* indices of houses and Breteau for *Aedes aegypti* were low, at 1.5% each, while the total breeding indexes were 0.5%, that was, less than one mosquito-positive recipient per household, with a maximum of four containers and minimum of zero.

General composition of mosquito fauna: spatial, macro and microhabitat, breeding sites

41 species were identified including: 2 species of Anophelinae and 39 species of Culicinae. Among the Culicinae there were: Aedini (three species), Culicini (22 species), Mansonini (four species), Sabethini (five species), Toxorhynchitini (one species) and Uranotaeniini (four species). Three species have been newly recorded for Ecuador (Table 1).

Three species (7%) were collected both in adult and immature stages, 11 (26%) species as immature in containers and 28 (67%) in adult phase by means of CDC light traps and mouth aspirators. Thirteen types of containers were artificial, and two were natural containers.

The species with the highest adult stage capture rate were *Culex* (*Melanoconion*) *spissipes* Theobald, *Anopheles* (*Anopheles*) nr. *mattogrossensis* Lutz & Neiva and *Coquillettidia* (*Rhynchotaenia*) *albicosta* Peryassú, while the most frequent in containers were: *Limatus durhami Theobald, Culex (Carrollia) bonnei Dyar,* Culex (*Car.*) *secundus* Bonne-Wepster & Bonne, *Culex* (*Culex*) *declarator* Dyar & Knab and *Wyeomyia melanocephala* Dyar & Knab. In phytotelmata only *Wy. melanocephala* showed the specificity for natural breeding sites in the Araceae family (*Xanthosoma* sp. and *Colocasia esculenta*

Spatial distribution / macrohabitat / Neighborhoods

A total of 25 species (62%) were collected only within the urban area, 11 (26%) both in the urban area and in the ecotone population-Biological Reserve, while five species (12%) were collected in the ecotone. Only three species (*Aedes aegypti, Culex quinquefasciatus* and *Limatus durhami*) were associated with the urban environments, while 93% were species of the jungle habitats (Table 3).

By neighborhoods, the greatest species richness was associated with the neighborhoods in central position (Barrio Central, August 8 and Pachakutik), decreasing the number of species towards the peripheries (Figure 2).

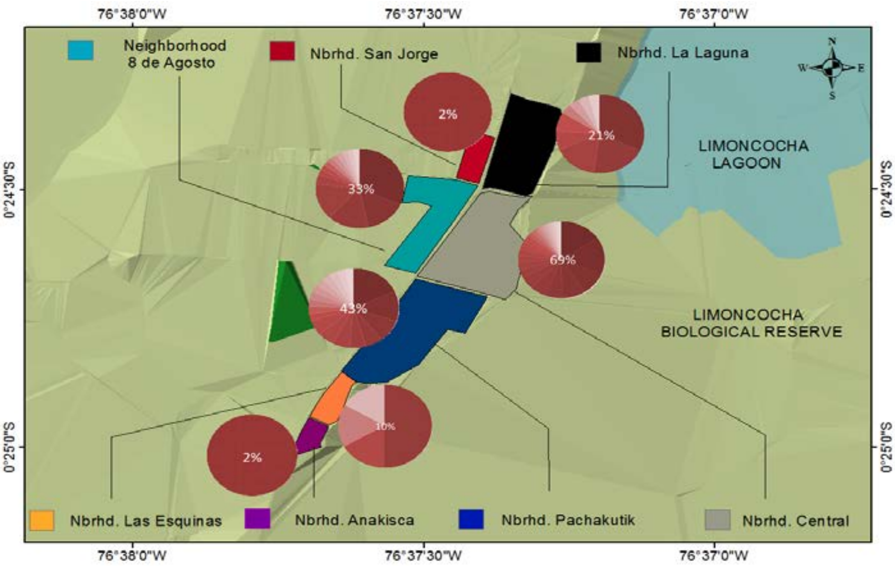


Figure 2. Spatial distribution of richness (percentages) and abundance of mosquitoes by neighborhoods (Nbrhd) in the town of Limoncocha.

Spatial distribution / microhabitat / breeding sites

The largest catch was made as the adult mosquitoes were caught in CDC light traps, while the artificial containers were of the greater importance (Table 1), and a significant percentage shared the housing and ecotone environments of the Natural Reserve, with the greatest species richness in the urbanized area (Table 2).

Rapid mosquito assessment and vulnerability to pathogen transmission in the urban area

In the locality of Limoncocha as urbanized environment (dwellings with the systematic division in seven neighborhoods, streets, churches, health centers, sports areas, shops), 35 species were collected plus one as unidentified (86%), while in the ecotone (Limoncocha Biological Reserve) 17 (40%) species were captured.

An evaluation of the entomological and socioeconomic risk factors for the transmission of arboviruses was carried out with the following results: 38% of the dwellings maintained Culicidae larvae, with *Aedes* home index IC = 1.56%, index of recipients IC = 2, 9% (1 positive to *Aedes* of 35 positives with water); Breteau index presented the same results as the Aedic index BI = 1.56%, with an average of 0.5 recipients per house.

Two types of breeding sites were determined including: the natural (11%) and artificial containers (89%). The total number of the artificial containers (positive and with water) was 31, of which 13 (42%) were useful (the storage of water for human consumption or for the use of domestic animals), 17 (55%) of waste or discarded and accumulated in patios and (3%) 1 ornamental (pupa of unidentified species). On the other hand, 4 natural breeding sites of the families Araceae (75%) and Poaceae (25%) were found.

In artificial breeding sites, the buckets (19%), pots (16%) and tires (13%) presented the highest percentages of the occupation with larvae or pupae of several mosquito tribes, followed by tanks (10%) and tubs (10%). The positivity of the occupation was between the mosquito species of the subfamilies Anophelinae and Culicinae. *Aedes aegypti* was only found in a barrel, used to store water, of a house in the Barrio Central neighborhood.

A total of 303 individuals belonging to the 41 identified species were captured. The highest total abundance was found in CDC light traps with 213 (70%) specimens, followed by the artificial containers with 75 (25%) specimens, 12 (4%) in natural containers and three (1%) individuals captured by the mouth aspirator. Among the breeding sites: pots (21%), buckets (18%) and Araceae plants (13%) had the highest abundance (Figure 4 and 6).

The highest species richness through capture was 31 (76%) species using plastic sucker (28 in artificial and three in natural containers) and 30 (73%) species by CDC light traps; while the lowest richness was two (5%) species captured by the mouth aspirator. The buckets (25%) and the pots (14%) were the artificial containers with the greater richness per recipient (Figure 3).

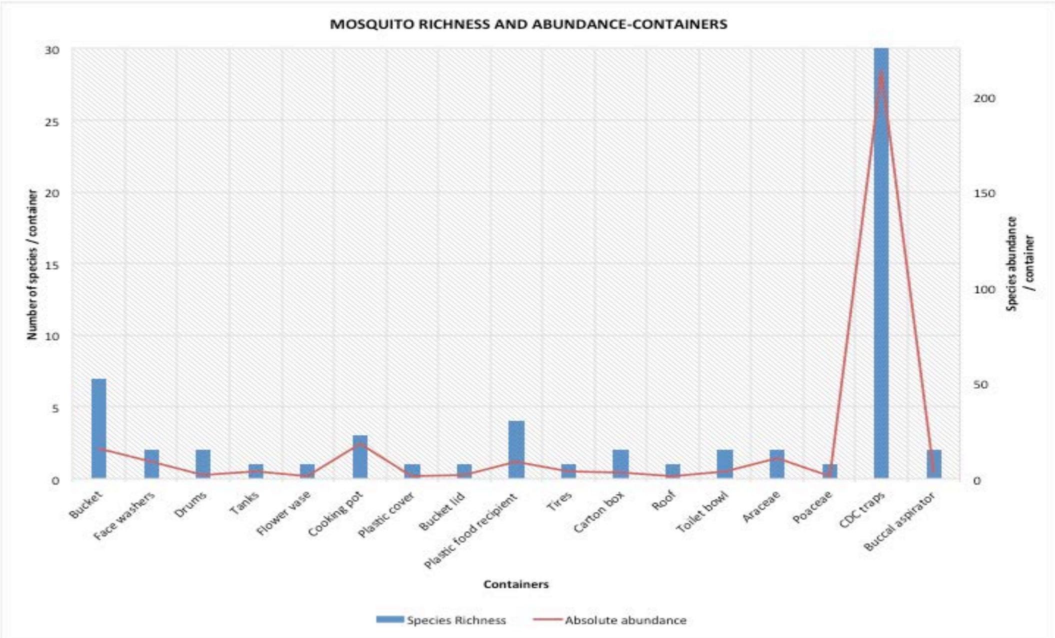


Figure 3. Richness and abundance of species per capture site/method (artificial and natural containers, CDC traps and mouth aspirator).

Analysis of classification of variables (Cluster and PCA)

According to the productivity of mosquitoes by neighborhoods (abundance of individuals per neighborhood), the Cluster (Figures 4 and 5) showed three productivity groups from the highest to the lowest, group 1: Barrio Central, August 8 and Las Esquinas, group 2: Pachakutik and La Laguna, and a third with the two ecotones and San Jorge as areas of the lower productivity.

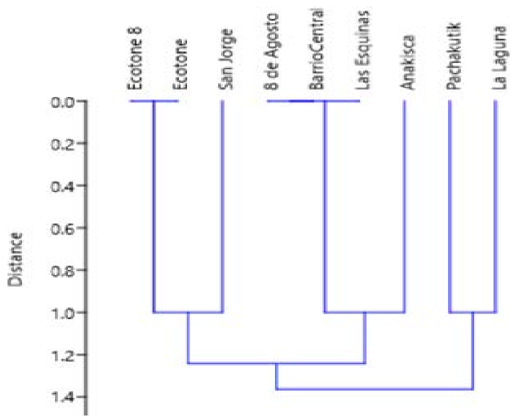


Figure 4. Cluster of neighborhoods by mosquito productivity

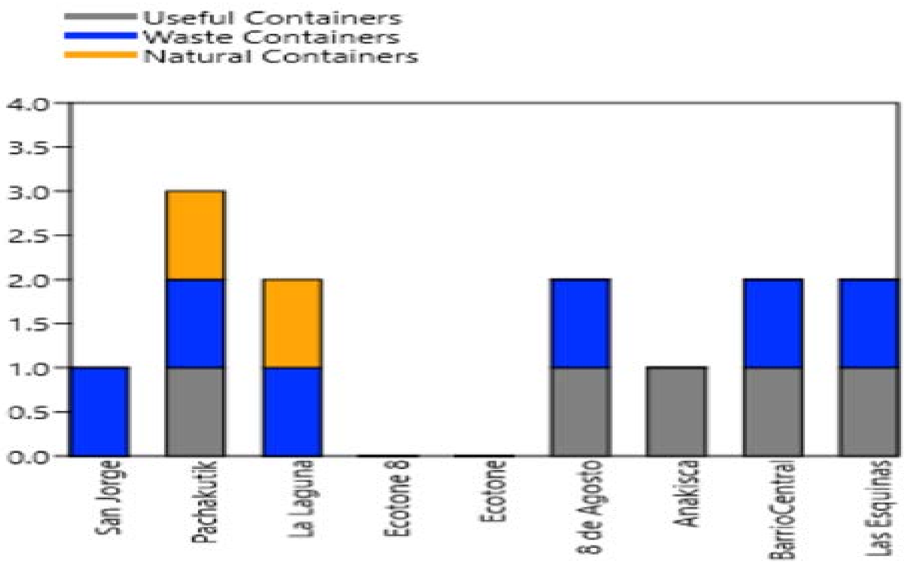


Figure 5. Positive breeding sites by neighborhood and kind of containers.

The Principal Component Analysis (PCA) with all the variables yielded five axes or components of which by their Eigen values, the first three explained 85% of the total variance, while the first two axes explained 64% of the variance (Table 3). Plotting the three components, the combination of the 1 Vs 2 components showed the best explanation of the variables that determined the eco-epidemiological patterns of Limoncocha (Figure 6).

Table 3. Eigenvalues yielded in the Principal Components Analysis (Figure 6).

Axis	Eigenvalues	%
1	0.467760	37.60
2	0.326320	26.23
3	0.264120	21.23
4	0.184040	14.79
5	0.001835	0.16

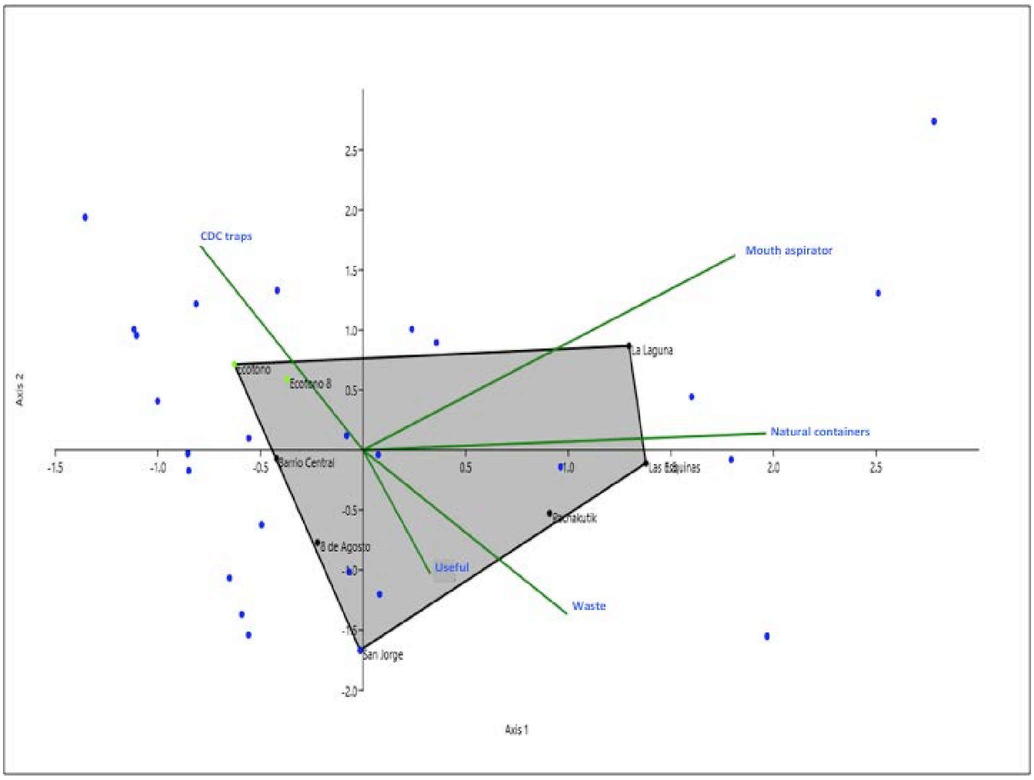


Figure 6. Principal component analysis (PCA). Component 1 Vs Component 2. Mosquito species by type of capture and neighborhood. In Blue letters: type of containers/capture, in black letters: Neighborhoods. Blue dots: species.

The axis 1 grouped the neighborhoods Pachakutic, Las Esquinas and La Laguna for the species of mosquitoes found in the containers (waste, useful and natural) and the mouth aspirator, while in the axis 2, the neighborhoods of Barrio Central, 8 de Agosto and San Jorge were grouped by catches of the adult mosquitoes in CDC light traps.

4. DISCUSSION.

The Limoncocha village has a water supply for drinking water (non-potable) that come from different sources including wells, groundwater, tankers, adjacent rivers and rainwater, which differentiates it from the water regime of the coastal areas of the country and Latin America with two well delimited seasons (dry and rain).

However, the fact that only 6 (10%) homes of 63 had a supply of potable water through the pipe line have caused the residents to store water from their different sources in the utility containers, buckets, barrels, tanks, and others of the smaller proportions.

A distinctive feature resulted in the most abundant type of storage container in Limoncocha, such as 5-liter buckets. The 200-liter barrels or barrels were the most important in the coastal areas and other Latin American countries [6,7,21]. These localities presented a marked seasonality and periods of drought that forced the community to store enough water for long periods of time, and for this reason they used the containers of the greater volume.

The case of Limoncocha differed in the social behavior of the storage, to have water supply by various routes, the main source were nearby bodies of water such as rivers and streams. This characteristic of having a safe and continuous source had a consequence that the inhabitants did not require large containers (drums or barrels of 200lt) and used the 5-liter buckets to collect water from wells, community outlets and nearby rivers, in which they constantly provided water.

This behavior led to the low accumulation of barrels or barrels in the houses, in which *Aedes aegypti*, -through the desiccation-resistant eggs attached to the walls-, has invaded different localities, as it happened to the wooden barrels during the colony in the transport of slaves from Africa to America.

On the other hand, although the garbage collection service was not markedly deficient (75% of the houses), this service did not collect the inorganic solid waste of various types found in the backyards of houses. Nor was there a disposal policy for these wastes. For these reasons, the highest percentage of the mosquito-positive containers was the waste (55%).

Despite these eco-social characteristics of Limoncocha, the *Aedes* index of 1.5% was below the estimated WHO value to avoid the epidemic outbreaks of diseases such as dengue, chikungunya and Zika, transmitted by Ae. aegypti. This premise allowed inferring that the cases of recent *Aedes*-borne diseases reported by the population were non-local transmission, infecting themselves outside the locality and probably in more active urban centers such as El Coca, Lago Agrio and Shushufindi. Likewise, the *Aedes* index suggested very low probability that any local or visiting individual might serve as a "seed" or source of the infection for the start of a local outbreak in Limoncocha, even with a high population density for both child and adult housing (3 and 3.8 / housing; respectively).

The 97% of the species were of jungle behavior, while only three species were urban, such as *Aedes aegypti, Culex quienquefasciatus* and *Limatus durhami*. This pattern of the presence / absence of the biodiversity of Culicidae with vector potential, suggested a mobility from reserve to the town, and that the greater vulnerability and risk of the population of Limoncocha was due to the transmission of potential circulating pathogens into the biological reserves and that could be translocated from it to the urbanized area.

The spatial distribution of the species in the stratification of the neighborhoods (Figure 3) showed how diversity was greater in the central neighborhoods, and was decreased towards the periphery of the town. This indicated that both useful and waste containers represented a source of significant breeding of jungle species in the locality and even more in the central neighborhoods. Likewise, it implied that there have been species that had the capacity as flying adults to reach the central point of the locality and achieve oviposit and development of their progeny in the houses. The species shared in the ecotones (Biological Reserve edge-urbanized area) and in the urbanized area supported this hypothesis.

This pattern suggested a high possibility of the species that could transport and transmit pathogens from the reserve to the urban areas and initiate an urban outbreak, through sylvatic-ecotone-urban cycles, as suggested for the Mayaro or Oropouche viruses, both probable public health problems in the near future [8,22,23].

This pattern was observed in the Principal Component Analysis (PCA, fig 6). The neighborhoods Pachakutic, Las Esquinas and La Laguna were characterized by the mosquitoes found in the containers (waste, useful and natural) and the mouth aspirator, while the neighborhoods Barrio Central, 8 de Agosto and San Jorge were characterized by the adult mosquitoes trapped in CDC light traps.

The PCA made it possible to discern between neighborhoods where some kinds of breeding sites or collecting methods predominated in order to establish spatial and stratified strategies to take efficient and effective control actions. In the same sense, the Cluster analysis and the bar graph of the containers associated with the neighborhoods (Figure 4 and 5) showed the neighborhoods with the highest productivity of larvae that coincided with those with the greatest diversity of the recipients and among the most productive, there were Pachakutic, Barrio Central and 8 de Agosto.

Assessment of mosquito diversity and epidemiological risk.

The capture of 41 species of Culicidae in three days of rapid and timely evaluation corresponded to the high diversity that Ecuador had. Until 2016, there was an underestimation of the Culicidae biodiversity for the country, with 243 identified species for the country [15], however recent works increased this figure to 253 [24-28]. In the present work, three new records for Ecuador were indicated: *Psorophora* (*Grabhamia*) *dimidiata*, *Uranotaenia* (*Uranotaenia*) *briseis* and *Coquillettidia* (*Rhynchotaenia*) *albicosta* (Table 1 and Table 2).

Among the six tribes identified in this study, four of them (Anophelini, Sabethini, Aedini and Culicini) belonged to the four most representative species of Ecuador that were directly related to the transmission of the pathogens and entomological risk, and the species that could act as epidemiological connectors from sylvan-rural cycles to urban cycles, generating an epidemiological problem [29] (Table 4).

Cx. quinquefasciatus is an important species that feeds on humans [30], and should be considered as a risk factor for the population. This species, associated with the transmission of filariasis, St. Louis encephalitis (SLEV) and West Nile virus (WNV), was found in the ecotone and within the urban area, and is nevertheless an urban species, has a worldwide distribution [15,21,31,32].

Arboviral infections have been found in four genera of the Sabethini tribe such as: *Johnbelkinia, Limatus, Trichoprosopon* and *Wyeomyia* [29,33,34]; and occurred in the locality. The majority of the species belonging to these genera were recorded in immature stages in artificial containers, with two of them (*Li. durhami* and *Wy. melanocephala*) occupying the highest rates of capture of individuals in breeding sites. This fact suggested the adaptation of the species to the urbanized environments since they have been usually associated with phytotelmata [33]. Several species of mosquitoes that carry arboviruses and affect people showed high rates of abundance in anthropogenic vessels and various types of capture.

5. CONCLUSIONS.

A direct relationship was found between the deficiencies of public services (drinking water supply and disposal of waste) as the cause of the breeding of different potential virus vectors. According to its composition, spatial distribution and abundance by the neighborhoods, it was possible to establish a medium-high degree of vulnerability and risk factors associated with the socio-environmental, entomological and ecological variables studied.

The results indicated that the area sampled in Limoncocha presented: the high biodiversity of the potential vectors of pathogens and the low density of the urban species. There was no environmental risk of the local transmission of the vector diseases transmitted by *Aedes aegypti.* The reported cases of diseases such as dengue, Zika and Chikungunya suggested that they have not been the product of the local transmission. However, the biological and epidemiological dynamics, coupled with the tourist and scientific mobilization of the area, contributed to an increase in the vulnerability in the local transmission of the pathogens from the sylvatic cycles, not yet demonstrated in the surrounding Reserve but with the evidence of the circulation in the Amazon [2,8,35,36].

The biological and socio-environmental variables (water supply and waste collection) must be considered for the development of the prevention strategies and the control of the possible emerging outbreaks of pathologies. Factors such as high species diversity, spatial distribution and increased container productivity in neighborhoods should be taken into account in order to prioritize and stratify zones of greater vulnerability. It would be important to work on effective public policies that guarantee coverage of the inorganic waste collection services and water supply by pipeline to the entire urban population of Limoncocha.

In the case of Limoncocha, the waste containers turned out to be the most important in the breeding of the potential vectors followed by the utilitarian ones, the product of the deficient service of collection and disposal of inorganic waste. The 5-liter buckets as utilitarian vessels were the main ones in the diversity of the larvae producers, being a consequence of a particular behavior of the population when having excess water sources, and at the same time the deficiency of the drinking water system by pipeline. The community, unlike the areas of coast with the marked seasonality (drought-rain), used these low-volume containers, being a possibly important factor in the low density of the vectors but not in the high diversity due to being immersed in a Biological Reserve.

The correlations of variables defined by the PCA and the spatial pattern of the diversity in the neighborhoods and ecotones defined the strata of vulnerability in the population of Limoncocha. The central districts had greater diversity than the peripheries, however, there was a pattern of possible mobility ecotone-periphery-center of the village that allowed inferring a high vulnerability to the translocation of the pathogens through jungle vectors, some of them with the behavior of the adaptation to the anthropogenic containers as new sources of breeding and their direct association to housing.

The low *Aedes* index suggested the low probability of the local transmission by *Aedes aegypti* of viruses such as Dengue, Chikungunya, both reported in Limoncocha, and for Zika virus, however, the explanation of this low density, even with the availability of the containers, was a question which was not resolved. One hypothesis to be tested was that the socio-economic behavior of the community and its little exchange with the large urban populations

nearby did not allow the entry of the invasive "seeds" such as *Aedes aegypti* eggs that have been resistant to the desiccation, and that facilitated their rapid and efficient invasion in new towns. It turned out to be unknown, if the little economic exchange out of the town and into the community was the limiting factor for its invasion and implantation, even though it has been a community with more than 60 years of foundation, and urban-rural characteristics were appropriate for its success invasion. The high rainfall in the area did not support the hypothesis of the temporary extinctions due to the periods of drought.
The vulnerability of the population was greater due to the possible translocation of pathogens from the reserve through jungle vectors, which mostly used waste containers as breeding sites, followed by the useful containers. To avoid this translocation, the intervention of the municipal authorities such as the Limoncocha population itself would be necessary in the elimination of the solid waste and the supply of a water system by reliable and uninterrupted pipes.
In this way, if the citizens of Limoncocha had a complete garbage collection service (organic and non-organic), there would not be a problem of storing waste containers in the yards. Equally, the provision of a reliable water system to the population by pipeline would eliminate the need to store water in useful containers.

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7. DECLARATION OF CONFLICT OF INTEREST

The authors declared the absence of any conflict of interest.

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Table 1. Checklist of mosquito species (larvae, pupae and adults), breeding sites and adult collecting methods in the Limoncocha village.

RECORDS		**Immatures stages (larvae and pupae)**																			**Adult**	
		Artificial containers															Natural containers					
		Useful				Ornamental	Waste/discharded										Soil	Vegetation				
Subfamily	**Species**	Bucket	Face washers	Drums	Tanks	Flower vase	Cooking pot	Plastic cover	Bucket lid	Plastic food recipient	Tire	Bottles	Carton box	Buckets	Roof	Toilet bowl	Pond	Araceae	Spathes-palms	Bamboo	CDC traps	mouth aspirator
Tribe	**Richness**	7	2	2	1	1 (lost)	3	1	1	4	1	0	2	0	1	2	0	2	0	1	30	2
Anophelinae																						
Anophelini	*Anopheles nr. mattogrossensis*																				X	X
	An. apicimacula																				X	
Culicinae	*Aedes aegypti*			X																		
Aedini	*Ae. fulvus*																				X	
	*Psorophora dimidiata***																				X	
Culicini	*Culex amazonensis*																				X	
	Culex bonnei	X					X	X					X			X						
	Cx. infoliatus	X					X															
	Cx. secundus	X	X							X												
	Cx. declarator	X			X					X					X							
	Cx. quinquefasciatus	X																			X	
	Cx. (Cux.) nine spp																				X	
	Cx. (Cux.) sp.10	X								X											X	
	Cx. ocossa																				X	
	Cx. spissipes																				X	
	Cx. five spp.																				X	
Mansoniini	*Coquillettidia albicosta***																				X	

	Cq. juxtamansonia																			X	
	Mansonia humeralis																			X	
	Ma. titillans																			X	
Sabethini	*Johnbelkinia longipes*																X				X
	Limatus asulleptus								X												
	Li. durhami	X	X				X			X	X		X			X					
	Wyeomyia melanocephala																X				
	Trichoprosopon compressum																		X		
Uranotaenini	*Uranotaenia briseis***																			X	
	Ur. calosomata																			X	
	Ur. geometrica																			X	
	Ur. lowii																			X	
Toxo-rhynchitinae	*Toxorhynchites* sp.			X																	

Note: ** New record form Ecuador.

Table 2. Spatial distribution of species by zone.

ECOTONE (5)	SHARING (11)	URBANIZED ZONE (25 + 1)
*Psorophora (Grabhamia) dimidiata***	*Anopheles (Anopheles) nr. mattogrossensis*	*Aedes (Stegomyia) aegypti*
Aedes (Ochlerotatus) fulvus	*Culex (Culex) quinquefasciatus*	*Culex (Aedinus) amazonensis*
Culex (Melanoconion.) sp. 1	*Cx. (Cux.) sp. 4*	*Anopheles (Anopheles) apicimacula*
Cx. (Mel.) sp. 5	*Cx. (Melanoconion) ocossa*	*Limatus asulleptus*
Mansonia (Mansonia) titillans	*Cx. (Mel.) spissipes*	*Culex (Carrollia) bonnei*
	Cx. (Mel.) sp. 3	*Uranotaena (Uranotaenia) calosomata*
	Cx. (Mel.) sp. 4	*Trichoprosopon compressum*
	*Uranotaenia (Uranotaenia) briseis***	*Cx. (Culex) declarator*
	*Coquillettidia (Rhynchotaenia) albicosta***	*Li. durhami*
	Cq. (Rhy.) juxtamansonia	*Ur. (Ura.) geometrica*
	Mansonia (Mansonia) humeralis	*Cx. (Car.) infoliatus*
		Johnbelkinia longipes
		Ur. (Ura.) lowii
		Wyeomyia melanocephala
		Cx. (Car.) secundus
		Cx. (Cux.) sp. 1
		Cx. (Cux) nine *spp.*
		Cx. (Mel.) sp. 2
		Toxorhynchites sp. 1

Table 3. Eigenvalues yielded in the Principal Components Analysis (Figure 7).

Axis	Eigenvalues	%
1	0.467760	37.60
2	0.326320	26.23
3	0.264120	21.23
4	0.184040	14.79
5	0.001835	0.16

Table 4. List of Culicidae species collected and their association with the transmission of emerging and re-emerging pathogens.

Family	**Species**	**Pathogen and / or disease**
Tribe		
Anophelinae	*Anopheles (Anopheles) nr. mattogrossensis**	Malaria
Anophelini	*An. (Anopheles) apicimacula*	Malaria
Culicinae		
Aedini	*Aedes (Stegomyia) aegypti*	DENV, VEEV, YFV
	Ae. (Ochlerotatus) fulvus	VEEV
	Psorophora (Grabhamia) dimidiata	VEEV
Culicini	*Culex (Aedinus) amazonensis*	VEEV, CARV, GMAV, CATUVs, BSBV, WYOV, ARUV
	*Culex (Carrollia) bonnei **	
	Cx. (Car.) infoliatus	
	*Cx. (Car.) secundus**	
	*Cx. (Culex) declarator**	SLEV
	Cx. (Cux.) quinquefasciatus	Wuchereria bancrofti, WNV, OROV, SLEV
	Cx. (Melanoconion) ocossa	VEEV
	*Cx. (Mel.) spissipes**	VEEV
	Cx. (Mel.) four *spp*	VEEV, WNV, WEEV
Mansoniini	*Coquillettidia (Rhynchotaenia) albicosta**	
	Cq. (Rhy.) juxtamansonia	
	Mansonia (Mansonia) humeralis	
	Ma. (Man.) titillans	VEEV
Sabethini	*Johnbelkinia longipes*	VEEV, MAYV, WYOV
	Limatus asulleptus	
	*Li. durhami**	MAYV, WYOV, CARV
	*Wyeomyia melanocephala**	CARV, WYOV
	Trichoprosopon compressum	
Uranotaeniini	*Uranotaenia (Uranotaenia) briseis*	
	Ur. (Ura.) calosomata	GMAV
	Ur. (Ura.) geometrica	
	Ur. (Ura.) lowii	

*most abundant species.

VEEV: Venezuelan equine encephalitis; WEEV: Wester equine encephalitis virus; OROV: Oropouche virus; WYOV: Wyeomyia virus; MAYV: Mayaro virus; YFV: Yellow fever virus; SLEV: St. Louis encephalitis virus; WNV: West Nile virus; CARV: Caraparú virus; ARUV: Aruac virus; GMAV: Guama virus; BSBV: Bush bush virus; CATUV: Catu virus; DENV: Dengue.