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Full Length Research Paper

Aedes aegypti larval index (Diptera: Culicidae) and its relation with the occurrence of Dengue and Chikungunya cases in the Province of Orellana. Ecuador

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The study of larval fluctuations of A*edes aegypti*, through entomological indicators (IE) define and reorient control actions. Such strategy, available in the surveillance and control program of *Ae. aegypti* allow to decrease the contact man-vector. The practical utility of housing index (IV), deposit index (ID) and Breteau index (IB) is evaluated with the occurrence of Dengue and Chikungunya cases, as well as determining the monthly fluctuation and differences between the three indexes, in the province of Orellana, Ecuadorian Amazon. Methods: This was a descriptive, field study and cross-sectional investigation. The indexes were obtained from the census and interventions made in housing inspections to 100% of the cases denounced during the epidemiological weeks 1-33 of 2015. The entomological indexes were analyzed from the averages and were compared according to cases, zones and months,through the analysis of variances, being adopted as level of significance "P" of values lower than 0.05. Results: When the three indexes were associated with dengue cases, the difference was not statistically significant (P> 0.05). ID recorded the highest average among the neighborhoods that recognized between (n = 3-4) Chikunguya cases; so this difference was statistically significant (P <0.05). No statistical association was found between the occurrence of cases for both arbovirosis with the indexes and sectorization by zones carried out in the study. Both IB and IV reached the highest average in the month of April and lower average in January, being such a statistically significant difference (P <0.05). ID recorded the highest average in the month of May and the lowest average in January, being the correlation statistically significant (P <0.05). The ID could be a good predictor of *Ae. aegypti* in cases of CHIKV.

Keywords: evaluation, entomological indexes, *Aedes aegypti*, dengue, chikungunya.

INTRODUCTION

Dengue Virus (VD) is considered by the World Health Organization (WHO) as an emerging disease (Clark, 1995). Chikungunya (CHIK V) since 2004, is a pathology that has caused major outbreaks, with considerable morbidity, suffering, laboral absenteeism, expenses to people and to health system, derived from hospitalization of the patients and / or necessary control campaigns of

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the vector, *Aedes aegypti* (Arredondo et al., 2016). Several strategies have been devised by International Organizations, even by each country, in order to reduce vector populations, morbidity and mortality for this cause (Pérez et al., 2016). The study of larval fluctuations of *Ae.aegypti*, through entomological indicators (EI), define and reorient control actions to replace eggs or adult collections. Usually, three indexes are used to record levels of infestation: the Household Infestation Index or Aedes housing Index (AHI), Recipients Index (RI), and Breteau Index (BI) (Romero et al., 2002; Sharma, 2001).

Larval indexes have been used for more than 60 years to estimate *Ae. aegypti* Linnaeus (Diptera: Culicidae) and in this way determine the possible risk of dengue transmission. Such strategy is available in the program of monitoring and control of the culicide allow to decrease man-vector contact Marquetti et al., 1999; Chadee and Rahaman, 2000).

The most common methodologies used by the Vector Control Programs are based in samples of Aedes larvae (Alves and Da Silva, 2001). The basic sampling unit, inspected systematically by vector control personnel in the house or buildings are empty containers or with water. In those items, they seek for larvae and pupae of mosquitoes and their exuvias (Strickman and Kittayapong, 2003). It is an easy-to-execute strategy, however, activities inherent to the treatment and control of a case involves the mobilization of human personnel, which usually requires a vehicle and logistics to carry out the activity.

However, there are discrepancies regarding the true usefulness of these indexes and whether they clearly define control strategies for epidemic and outbreak prevention. In this regard, Fernández and Iannacone (2005) indicated that three indexes showed differences during the time the study was carried out, emphasizing that only a linear correlation was found between (AI) and dengue cases. On the other hand, Méndez Espinosa and Ramos Peña (2003) recommend that studies of larval indexes should be expanded, since the larger the areas examined, the greater the association between cases of dengue and the presence of larvae. Similarly, the authors indicate an entomological indicator that must be considered in the epidemiological surveillance since it was proved an association between the variables of the study in the Municipality of Monterrey in Mexico, since they reported more cases of Dengue when more areas were examined. Similarly, Espinoza Gómez et al. (2001) reportedthatHI showed association with the Household Index (HI) as a good estimator of *Ae. aegypti* infestation.

In Ecuador, despite permanent activities for the prevention and control of Chikungunya and Dengue Fever, it was found in the Study Area that the (IB) was 18.6% exceeding the range limit (3-5%) despite of fumigation campaigns were carried out in-house. To that effect, a total of 1226 fumigated houses were registered, 9 closed and 4 reluctant, protecting 5062 inhabitants covering an area of 126 blocks (within the period from June 01 to 05 2015 of District 22D02 Orellana - Loreto Health MSP, 2015 Revisar).

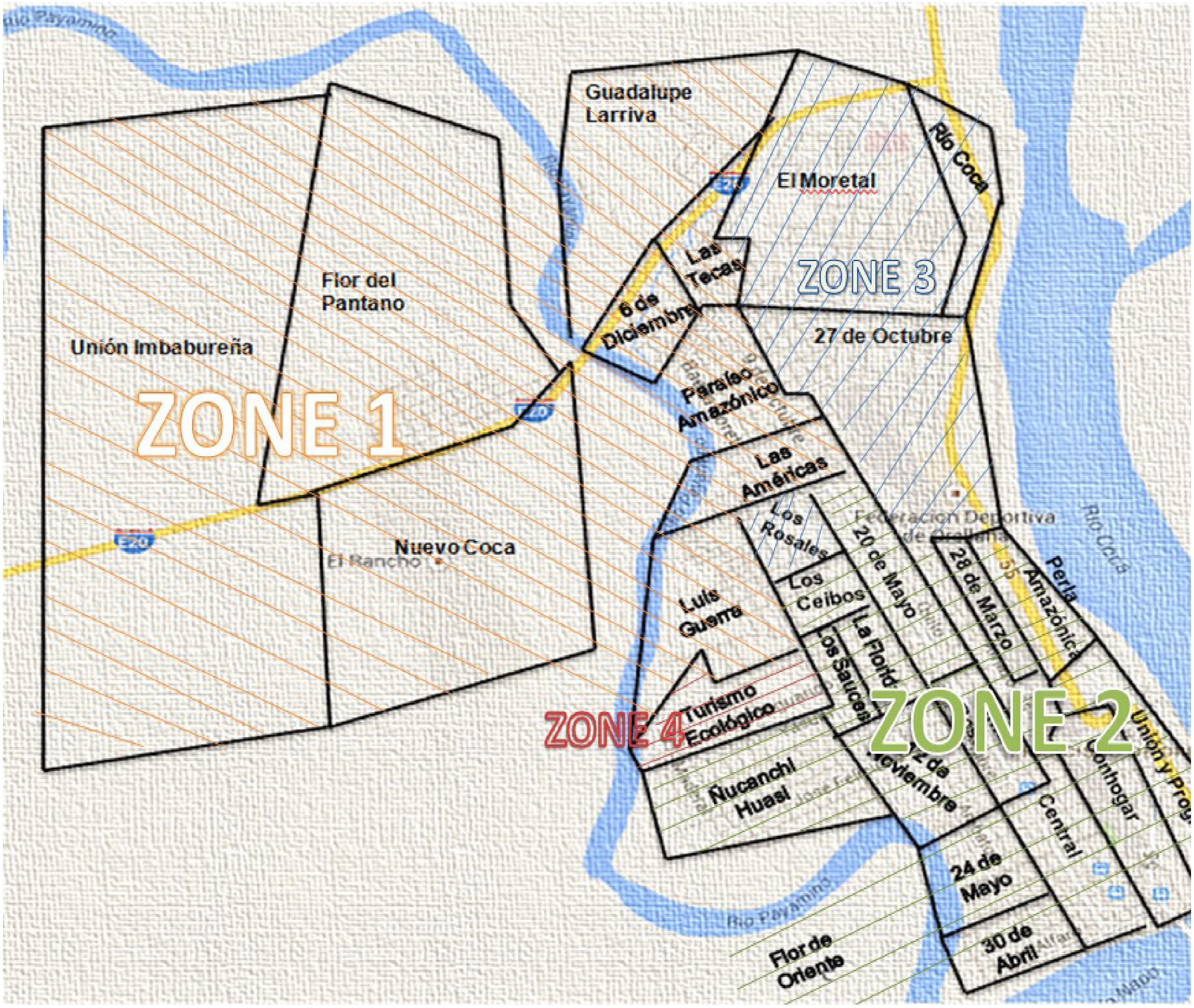
Considering the importance for public health that signifies the practical and economic utility of the realization of entomological indicators, so this strategy can truly represents an effective measure to define risk areas where the vector is present. In this sense, the objective of this study is to evaluate and estimate the practical utility of the Breteau index (IB), Housing index (HI) and deposit index (DI) with the occurrence of Dengue and Chikungunya cases and determine the monthly fluctuation and the differences between the three indexes, in the province of Orellana, Ecuadorian Amazon.

METHODOLOGY

It was a field study, descriptive, prospective, cross-sectional investigation. It was performed in the Province of Orellana, Canton Puerto Francisco de Orellana (Coca) located to the Northwest of Ecuador. The study area is located at the coordinates 0 ° 56'00 "S 75 ° 40'00" O (ECY, 2016). The cantonal area involves a total area of 7,047 km2 (704,755 ha). The Canton limits to the North with the Canton La Joya de los Sachas (Province of Orellana) and the Cantons Cascales and Shushufindi (Province of Sucumbíos); to the south with the Cantons Arajuno (Province of Pastaza) and Tena (Province of Napo), to the East with the Canton Aguarico (Province of Orellana) and to the West with the Canton Loreto (Province of Orellana) and Tena (Province of Napo) (Plan de Desarrollo y Ordenamiento Territorial Municipal de Francisco de Orellana, 2014).

The study area belongs to a warm humid tropical climate, with average annual temperatures of 26.19 ° C and rainfalls reaching to 3,126.9 mm. With a relative humidity of 81%, the altitude ranges from 100 to 720 msnm (Llangari et al., 2016).

The study period covered the months of January to August 2015. The indexes were obtained from the census and interventions during the housing supervision from 100% of cases denounced during the epidemiological weeks 1-33 of that year. In order to this end, the province was divided into districts and these were grouped into zones (Figure 1). Understanding "Neighborhood" as "a recognizable territorial unit, and the community that composes it (Blanco-Moya, 2015). The limits were determined for administrative, political, and other reasons (DRAE, 2017). For this, the Province was divided into four zones (1-north, 2-south, 3-east and 4-west). Zone 1: was confirmed by the neighborhoods Americas, Amazonia Paradise, Julio Llori, Nuevo Coca, Luis Guerra, Las Tecas, December 6, Guadalupe Larriva, Imbabureña Union and Flower of the Swamp; Zone 2: constituted by the neighborhoods Union and Progreso, Conhogar, Amazonian Pearl, March, Central District,



Map showing the Zones of vector influence in El Coca, Orellana-Loreto. Ecuador. 2015.
Source: Research data (Velásquez et al., 2015)

Santa Rosa, May 20, April 30, May 24, November 12, Florida, Los Sauces, Los Ceibos, ÑucanchiWasi, Machala and Flor de Oriente. Zone 3: 27 de Octubre, El Moretal, Coca River and Los Rosales and finally, the Zone 4: composed by the neighborhood, Ecological Tourism.

The Analysis Unit was represented with the cases denounced from all the housing units. The data used by the health workers (technicians) of the Vector Control Program (SNEM, 2013) was used as a support material for the purpose of collecting indexes, housing census and interventions. Program indicators were used, allowing to stratify the entomological risk in the study areas by months and seasons. The indicators used were: Housing Index (HI): (N° of positive houses / N° of houses inspected) x 100; Index of Deposit (DI): (No. of positive containers / Number of containers inspected) x100 and Breteau Index: (BI): (No. of positive containers / No. of houses inspected) x100 (OPS, 1995).

Statistical analysis

The entomological indexes were analyzed from the averages of the values obtained by field workers. The Standard Error was calculated and compared, according to Dengue, Chikungunya, zones and months of the study, through the Analysis of Variances, adopting values of less than 0.05 as a level of significance "P".

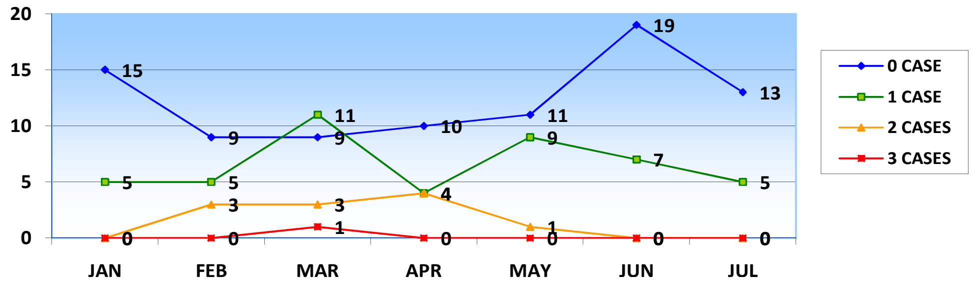


Figure 1. Fluctuation of DV cases. Orellana. Ecuador. EW 1-33. 2015.
Source: Research data (Velásquez et al., 2015)

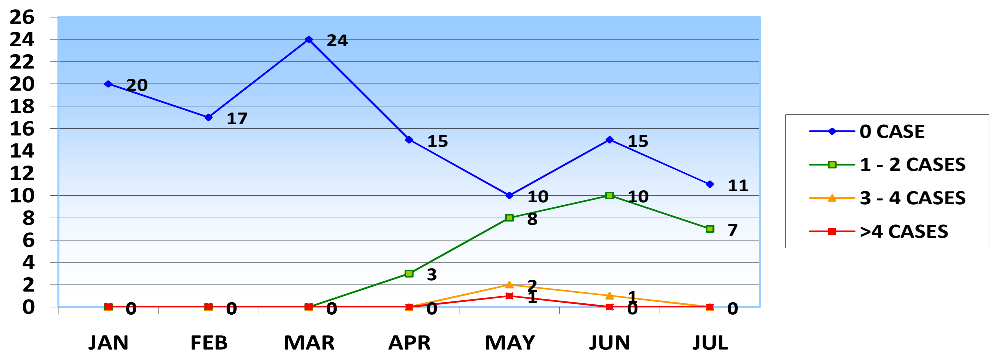


Figure 2. Fluctuation of CHIKV cases. Orellana. Ecuador. EW 1-33. 2015.
Source: Research data (Velásquez et al., 2015)

Table 1. Fluctuation of three entomological indexes: HI, DI and BI according to the number of cases of Dengue Virus. Orellana. Ecuador. Semana Epidemiologica EW 1-33. 2015.

Dengue cases (during the ew)	**0 (n=76)** $\overline{X} \pm Es$	**1 (n= 23)** $\overline{X} \pm Es$	**2 (n=4)** $\overline{X} \pm Es$	**3 (n=1)** $\overline{X} \pm Es$	**F**	**P value**
Breteau Index	5,31 ± 0,61	4,93 ± 0,86	5,38 ± 2,37	8,3	0,16	0,9248
Dengue cases	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	F	P value
Housing Index	3,34 ± 0,33	3,0 ± 0,44	3,38 ± 1,54	4,4	0,15	0,9304
Dengue Cases	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	F	P value
Deposit Index	0,89 ± 0,14	1,47 ± 0,42	0,53 ± 0,23	0,7	1,11	0,3503

Source: Research data (Velásquez et al., 2015)

RESULTS

Sectors with single cases of dengue were more regular 31.94% (n = 46). Evident in months of March (n = 11) and May (n = 9). Sectors with (n = 2) cases represented 7.64% (n = 11) being more frequent in April (n = 4). (Figure 1)

Figure 2 shows the frequency of 1 and 2 cases of Chikunguya representing 19.44% (n = 28), being more frequent in the month of June (n = 10) followed by the month of May (n = 8). The sectors with 3 and 4 Chikunguya cases represented 2.08% of the cases (n = 3), being more recurrent in the month of May (n = 2).

The Breteau Index (BI) and the Housing Index (HI) registered the highest average among the localities that presented (n = 2) dengue cases, which was considered in the moderate risk range; the highest value of this index was recognized in the neighborhood that reported (n = 3) dengue cases. However, this difference was not statistically significant (P> 0.05). In the case of DI, the highest average was observed in the neighborhoods that registered (n = 1) dengue cases; however, this difference was not statistically significant (P> 0.05). (Table 1).

Table 2. Fluctuation of three entomological indexes: HI, DI and BI with cases of Chikungunya Virus. Orellana. Ecuador. 2015.

Chikunguya cases (during the ew)	**0 (n=88)** $\overline{X} \pm Es$	**1 - 2 (n= 12)** $\overline{X} \pm Es$	**3 – 4 (n=3)** $\overline{X} \pm Es$	**>4 (n=1)** $\overline{X} \pm Es$	**F**	**P value**
BreteauIndex	4,87 ± 0,51	6,95 ± 1,6	9,1 ± 4,92	7,6	1,32	0,2715
Chikunguya Cases	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	F	P value
HousingIndex	3,08 ± 0,29	4,5 ± 0,767	3,87 ± 1,62	3,9	1,02	0,3874
Chikunguya Cases	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	F	P value
DepositIndex	0,80 ± 0,12	1,71 ± 0,59	3,23 ± 2,0	3,9	5,90	0,0009*

Source: Research data (Velásquez et al., 2015)

Table 3. Fluctuation of three entomological indexes: HI, DI and BI according to the zones in the study. Orellana. Ecuador. SE 1-33. 2015.

Zones	**1 (n=34)** $\overline{X} \pm Es$	**2 (n= 54)** $\overline{X} \pm Es$	**3 (n=12)** $\overline{X} \pm Es$	**4 (n=4)** $\overline{X} \pm Es$	**F**	**P value**
Índice Breteau	6,14 ± 1,01	5,04 ± 0,65	3,7 ± 0,87	5,35 ± 1,29	0,78	0,5091
Zones	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	F	P value
HousingIndex	3,70 ± 0,51	3,09 ± 0,36	2,58 ± 0,65	4,2 ± 1,47	0,77	0,5108
Zones	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$	F	P value
DepositIndex	1,15 ± 0,28	0,97 ± 0,19	0,65 ± 0,31	1,35 ± 0,82	0,42	0,7367

Source: Research data (Velásquez et al., 2015)

The BI recorded the highest average among the districts that registered (n = 3-4) cases of Chikungunya, being considered, this in the Moderate Risk range. The lowest average was evident in those neighborhoods without Chikungunya cases; being this difference not statistically significant (P> 0.05).

The (HI) also recognized the highest average among the neighborhoods that registered between (n = 1-2) Chikungunya cases; however, this difference was not statistically significant (P> 0.05). Finally, the DI recorded the highest average among the neighborhoods that recognized between (n = 3-4) Chikungunya cases; so this difference was statistically significant (P <0.05) (Table 2).

According to the evaluated zones (Table 3), the BI recorded the highest average in Zone 1 and the lowest average in Zone 3; however, this difference was not statistically significant (P> 0.05). The (HI) showed the highest average in Zone 4 and the lowest average in Zone 2; however, this difference was not statistically significant (P> 0.05). The DI obtained the highest average between the districts of Zone 4 and the lowest average in Zone 3; being such difference not statistically significant (P> 0.05).

According to the months of this period, the BI registered the highest average in the month of April and the lowest average in the month of January, being the statistically significant difference (P <0.05). (HI) also showed the highest average in the month of April and the lowest average in the month of January, observing that this difference was also statistically significant (P <0.05). The DI, revealed the highest average in the month of May and the lowest average in the month of January; being this difference statistically significant (P <0.05).

Table 4. Variation of the entomological indexes HI, DI and BI between the dry and rainy periods. Orellana-Loreto. Ecuador. EW 1-33. 2015.

Entomological Index		**Breteau Index**	**Housing Index**	**Deposit Index**
Months	n	$\overline{X} \pm Es$	$\overline{X} \pm Es$	$\overline{X} \pm Es$
January	18	1,86 ± 0,44	1,16 ± 0,25	0,20 ± 0,04
February	13	3,43 ± 0,61	2,33 ± 0,39	0,32 ± 0,06
March	14	3,95 ± 1,02	2,94 ± 0,79	0,36 ± 0,09
April	14	10,25 ± 1,71	5,91 ± 0,89	1,01 ± 0,18
May	14	7,41 ± 1,0	4,26 ± 0,37	4,26 ± 0,37
June	21	5,78 ± 1,28	3,8 ± 0,64	0,63 ± 0,12
July	10	4,47 ± 1,26	2,63 ± 0,75	0,48 ± 0,13
F		6,12	6,33	71,48
P value		0,0000*	0,0000*	0,0000*

Source: Research data (Velásquez et al., 2015)

DISCUSSION

Dengue in any of its forms of presentation continues to be a public health problem that is difficult to control (PAHO, 2010). In addition, there is another emergent arbovirus disease in Ecuador, such as the fever caused by the virus Chikungunya (IMSS, 2014).

In relation to Dengue virus, Orellana is one of the endemic areas, where it is demonstrated by the Old National Service of Metaxenic Diseases (SNEM, 2013) as one of the first three provinces with a disease burden in the last 10 years, demonstrated by the variability of the aedical indexes and the population density of the vector in this territory. In addition, it is evidenced by daily morbidity records (MSP, 2015) and by the representation of work absenteeism of those who suffer from this disease (Clark et al., 2005).

Chikungunya fever is a recent infection in the Americas; first reported cases were in December 2013, in contrast to the continents of Asia and Africa, which have reported cases of this disease since 1952, where the virus was identified and isolated in Tanzania (Staples et al., 2009), which probably requires greater knowledge and understanding due to the need to identify new specific risk factors to each particular area where it is present.

In this investigation, cases of DV were more frequent as unique, isolated cases, during the months of March and May. On the other hand, cases of CHIKV predominated frequently isolated or in number of two, mainly, in the month of June.

The data shows an increase in the Breteau Index, making the occurrence of cases when the field work has not been followed and exceeds two months. Uribarren Berrueta (2015) indicates in Mexico, that in the epidemiological week Number 15 (May) there was an important increase of notified probable cases of Dengue, which were later confirmed as CHIKV. It also prevailed in Ecuador, from April to July throughout the country, according to a report issued in the Epidemiological Surveillance Bulletin (MSP, 2016). It should be noted that the highest number of cases of Chikungunya virus fever, were perhaps due to the impossibility of vector control of all cases demanded by the epidemy.

When comparing the Breteau Index for both arbovirosis, it was observed that for both DV and CHIKV, the risk was classified as Moderate (5-10%). In the case of Dengue, it demonstrated its highest average for (n = 2) cases. In Chikungunya virus, BI had the highest average when the occurrence was 3-4 cases. In this case, the value of (p> 0.05). There was no correlation between the number of cases of arbovirosis in the study and the values of the averages obtained. According to the Brazilian Ministry of Health (2015)estimates of the BI will be subject to sampling errors, translated by confidence intervals that are adopted, which provide indications on the accuracy of the estimates. Operationally, it is easier to adopt a single sample size in the diagnosis of larval density, performed in different months and cantons (health districts) and to determine the number of properties that must participate in sampling, regardless of the expected BI. If the area of the study is smaller in relation to the size of the fixed sample, it is possible to apply a correction andwith it, reduce the number of houses affected with the sample. In our study, the accuracy was 95%, adopting values of less than 0.05 as the "P" significance level.

Regarding to Housing Index, as a result of the inspection of each case diagnosed as Dengue Virus, the highest average was found in the neighborhoods that reported (n = 3) cases of DV. Likewise, for Chikungunya fever it was recognized as the highest average value among the neighborhoods that registered between (n = 1-2) cases; so no statistically significant difference was found (P> 0.05). We consider, in a particular way that this could obey to great variability in the housing index between localities. In this research motivated to the particular characteristics of each zone in study, influenced by environmental factors in some, in others to the inadequate treatment of solid wastes, accumulation of junk (useless breeds) which facilitates the growth of vector, evidenced after inspection.

The Deposit Index registered the highest average among the districts that registered (n = 1) dengue cases; However, for CHIKV, it recorded the highest average among the localities that registered between (n = 3-4) cases, this difference being statistically significant ($P <0.05$). Such result could indicate that as lower the average deposit rate, with number of DV cases equal to 1, the greater the likelihood of CHIKV cases occurring. This observation is probably motivated by the lack of dissemination of preventive measures to combat vector breeding sites and the impossibility of timely vector control. Also, if CHIKV is circulating concomitantly with DV, due to the proximity between the zones, so the importance of timely measures of epidemiological surveillance, once we have information from the first case. Now, in those cases of CHIKV whose averages were higher for the ID, it would possibly be linked to the temporary permanence of viruses in the area, associated to several factors indistinct to those described by the vector or host (relative humidity of the environment, vegetation, temperature, age, immunity, among others)

We did not find an association between the three indexes (BI, HI, DI) obtained for the Province in general with the occurrence of Dengue and Chikungunya cases. Neither a correlation between the indexes obtained for each zone with the presence of cases in the same. This result coincides with Fernández and Iannacone (2005) who did not observe significant differences between the 11 areas studied for HI and BI when evaluating the Variations of three larval indexes of *Ae.aegypti* (L.) (Diptera: Culicidae) and their relationship with dengue cases in Yurimaguas, Peru. However, Méndez-Espinoza and Ramos Peña (2003) in a study aimed about the association of larval index of *Ae.aegypti* and dengue in the state of Nuevo León (Mexico) found that only in the municipality of Monterrey with 15 cases showed a statistically significant association between dengue cases and larval indexes.

On the other hand, in Orellana, more cases had been reported together with the proximity between the zones, facilitated by travel, communication, transport and mobility of people between them, could allowed to transmit the dynamics of the transmission but not the association between the indexes, since the areas examined were greater. However, different geographic levels are used to calculate the indexes in the studies, and the appropriated level for entomologic indexes is in itself an issue of debate (Llangari et al., 2016).Fernandez and Iannacone (2005) point out that the transmission and presence of cases can occur indistinctly in any of the zones, even though the rates are low. Therefore, monitoring and control should not be focused only in those areas where epidemiological indicators are high. We agree with the author that areas with low HI rates will not necessarily have a lower incidence of cases.

When evaluating the three indexes, according to the months of the period evaluated, it was observed that the BI and the HI registered the highest average in the month of April. This period represents the winter stage of Ecuador (November-April) where vector control work intensifies; After that time of year, vector control activities are reduced, so only sporadic cases are inspected. In that investigation, the association of these indexes were statistically significant ($P <0.05$). Although it is difficult to establish comparisons between the time elapsed between the research presented and the results, Fernandez and Iannacone (2005) emphasize that the three indexes showed a tendency to decrease in the period (April 2000 to December 2000). This result does not coincide with the data obtained in the present investigation, could be due to the culmination of the rainy season in the territory, where an increase of the precipitations in that month was evidenced.

The Deposit Index, on the other hand, showed the highest average in the month of May and the lowest average in the month of January; Being this difference statistically significant ($P <0.05$). This result could indicate that the indexes were markedly influenced (averages) by the influence of the precipitations and temperature as expected. Stein et al. (2005) indicate precipitation as the main factor to determine the presence of the vector and the epidemiological risk of the disease with a correlation value of $r = 0.57$ ($p <0.05$), which agrees with the occurrence of infected persons.

We coincide to indicate that the correlation between the entomological indexes and the incidence of dengue cases is inconsistent, underestimated and poorly defined at certain times (Tun-Lin et al., 1996). At the present time, whether they are standardized in the country, the system of registration of the indexes under study could be obtained with a more precise information on the real practical usefulness in the Program of Control of vectors, allowing this to clarify and define the epidemiological scenario in a situation of epidemy and outbreaks.

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