

Prevalence of larvae of potential yellow fever vectors in domestic water containers in south-east Nigeria

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The seasonal variation in prevalence of Aedes (Stegomyia) mosquitos breeding in peridomestic water containers was assessed in an urban quarter of Enugu, Nigeria, and in two rural villages located among forest relicts in the neighbouring Udi Hills. A large number of earthenware pots, most of which contained water in the wet season, were present in the compounds around houses. Monthly determinations of the presence or absence of Aedes larvae in these containers were made for 13 consecutive months. The average Breteau index (positive containers per 100 houses) for A. aegypti during the 7-month wet season was 53 in one of the villages and 76 in the other, suggesting a high risk of yellow fever transmission; the dry-season averages were 11 and 23. In the urban quarter the wet-season average was 29; the dry-season average was 4.7, a level at which transmission is unlikely to occur. A. luteocephalus were occasionally found in containers in both the urban and rural localities, and A. africanus larvae occurred in one of the villages. Although Culex larvae were common, mixed infestations of Aedes and Culex were so uncommon that the simplified "single larva" method of sampling for Aedes gave similar results to the conventional method. The multiplicity of peridomestic containers in this part of Nigeria made the container index inadequate as a measure of larval density.

The World Health Organization Arbovirus Vector Research Unit was established at Enugu, Nigeria, in 1973, and initiated a study of the prevalence of the various species of mosquito vectors in the Jos plateau area where there had been a yellow fever epidemic in 1969 (4).

This paper presents the findings on the density and seasonal prevalence of *Aedes* infestations in peridomestic water containers in two rural communities in the Udi Hills (06° 29' N, 07° 23' E) near Enugu, and in one urban area of Enugu (06° 27' N, 07° 29' E). The methods used in larval surveillance are also discussed, particularly the validity of a simplified method of sampling infested containers, the implications of the container index and other indices in an area where the number of outdoor domestic containers is very large, and the relationship of the indices obtained to the likelihood of yellow fever transmission in the area.

THE STUDY AREA

The climate in the area around Enugu is marked by two well defined seasons, wet and dry. The rainy season extends from mid-March to mid-October, the annual rainfall being nearly 1500 mm. The average maximum temperature ranges from 29 °C in August to 34.5 °C in February, while the average minimum ranges from 20 °C in January to 23.3 °C in March. The dwellings in the two rural communities surveyed, Egede and Abor, are dispersed within modified forest relicts on the Udi Hills, at an altitude of approximately 500 m above sea level. The vegetation is characteristic of the biotypes between the lowland rain forest and the southern Guinea savanna, and is designated as derived savanna forest because it has been modified by cultivation. In Enugu (altitude about 350 m), the densely populated quarter of Ogui was surveyed.

There is a public piped-water system in Ogui, but the supply is usually irregular and often inadequate. The rural communities rely completely on rainwater stored in clay pots or on water collected from a stream about 6 km from the villages. The average number of family members in each compound ranged from 30 in Ogui to 4 in Egede. Chicken were the most common domestic animals at an average of 1.5 per person. Each dwelling had its own compound surrounding it.

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METHODS

The usual method of making a larval survey of *Aedes aegypti* and other *Stegomyia* vectors is to examine all the containers of fresh water or drinking water associated with houses for the presence of the larvae. In the conventional method, up to 10 larvae per container are examined to verify the species; in the more rapid "single-larva-per-container" method (10) only one larva is taken for identification. The results of the survey may be expressed as one of three indices of vector density (3, 12) as follows:

—container index: percentage of water-holding (wet) containers that are positive;

—house index: percentage of houses that have positive containers;

—Breteau index (2): number of positive containers per 100 houses.

For purposes of comparison and mapping,^a any or all of these indices may be converted to a single WHO density figure on a scale from 1 to 9 by the use of conversion factors^b derived from a large body of accumulated data over the global range of *A. aegypti* (3, 12).

At each locality in the Udi Hills and Enugu, 30 selected compounds were assessed for larval incidence once a month. Only outdoor containers were examined since there were few indoor water-holding containers in the rural communities, and no larvae were detected in them. Up to 10 larvae were collected from each positive container and placed in separate vials, as in the conventional larval survey. Following the identification of the larvae a separate record was made of the first larva picked out by the microscopist from the collection vial, in order to compare the conventional larval survey with the "single-larva-per-container" method (10). The surveys were carried out over 13 months from November 1974 to November 1975.

In a special experiment to ascertain the effect on the house index and Breteau index of the number of containers sampled, 40 compounds were selected, each with more than 25 outdoor containers, using houses in the village of Ukana (06° 30'N, 7° 23'E) to supplement those found in this category in Egede and Abor. Every container in each compound was numbered, and groups of 5, 10, 15, 20, 25, and more containers per compound were selected, according to the following scheme:

Group	No. of containers	Container no.
A	5	1, 6, 11, 16, 21
B	10	A plus 2, 7, 12, 17, 22
C	15	B plus 3, 8, 13, 18, 23
D	20	C plus 4, 9, 14, 19, 24
E	25	D plus 5, 10, 15, 20, 25
F	Unknown	E plus those pots additional to 1-25.

The pots were examined once a month from March to November, i.e., for the duration of the wet season.

RESULTS

Breeding sites

In all three localities, earthenware pots were the most common type of water container, comprising 96% in Ogui and 100% in Abor. Of all the containers in the house and compound, at least 95% were out-of-doors in the rural communities, while in urban Ogui 49% were indoors. The mean number of domestic water containers per compound ranged from 19 in urban Ogui to 23 and 30 in rural Abor and Egede, respectively (Table 1), and varied by less than 5%

Table 1. Percentage occurrence of mosquito species in larvae-positive outdoor containers

Mosquito species	Urban		Rural	
	Ogui	Egede	Abor	Mean
<i>A. aegypti</i>	68.5	7.5	10.4	8.95
<i>A. africanus</i>	0.0	0.1	0.0	0.05
<i>A. luteocephalus</i>	1.9	0.3	0.3	0.3
<i>A. longipalpis</i>	0.0	0.2	0.0	0.1
<i>A. apicoargenteus</i>	0.0	2.5	0.7	1.6
Two <i>Aedes</i> spp. together	2.8	0.8	0.3	0.55
Three <i>Aedes</i> spp. together	0.0	0.1	0.0	0.05
Total for <i>Aedes</i>	73.2	11.5	11.7	
<i>Aedes</i> and <i>Anopheles</i> spp. together	0.0	0.5	0.1	0.3
<i>Aedes</i> and <i>Culex</i> spp. together	5.5	1.5	2.4	1.95
<i>Culex</i> spp. only	21.3	77.1	81.8	79.45
<i>Anopheles gambiae</i> only	0.0	8.3	3.2	5.75
<i>Eretmapodites chrysogaster</i>	0.0	0.1	0.0	0.05
<i>Culex</i> and <i>Anopheles</i> together	0.0	1.1	0.8	0.95
No. of larva-positive containers	108	1792	1223	
No. of containers per compound	19	30	23	
No. of containers per capita	0.61	7.82	2.46	

^a WHO computer survey of *Stegomyia* mosquitos. WHO unpublished document VBC/73.11, 1973.

^b BROWN, A. W. A. Surveillance system for *Aedes aegypti* and related *Stegomyia* mosquitos in terms of density. WHO unpublished document WHO/VBC/73.464, 1973.

from one season to the other. The mean number of containers per person was 0.61 in Ogui, 2.5 in Abor, and 7.8 in Egede. In Ogui the percentage of containers that were wet remained fairly constant all year, but in Abor and Egede the percentages increased as the rainy season advanced, those for the early wet-season month of May being respectively four and nine times higher than they had been in February (Fig. 1).

Species distribution

Of the 108 water pots found to contain mosquito larvae in Ogui, 68.5% were positive for *A. aegypti* alone (Table 1). The only other *Aedes* species represented was *A. luteocephalus*, which occurred alone in 1.9% of the positive containers and together with *A. aegypti* in 2.8% of them. Altogether, 73% of the larvae-positive containers were inhabited by one or other of these two species.

In the two rural communities, where *Culex* spp. predominated, *A. aegypti* was again the most common *Aedes* (8–10%) in the 3015 larvae-positive containers. The next commonest *Aedes* species were *A. apicoargenteus*, *A. luteocephalus*, *A. longipalpis*, and *A. africanus*. *A. vittatus* was not found in any of the three localities surveyed.

C. pipiens fatigans comprised 53.3% of the *Culex* species infesting containers in Ogui, while in the rural villages *C. duttoni* comprised 37% of the *Culex*, followed by *C. tigripes* (29.7%), *C. decens* (28.3%),

and *C. nebulosus* (4.6%). The number of containers infested with other *Culex* species (*C. horridus*, *C. perfuscus*, *C. moucheti*) was less than 1.0%. The anopheline mosquitos found in the rural areas, infesting nearly 4% of the larvae-positive containers, were of the *Anopheles gambiae* complex. In these villages, 2.0% of the positive containers were infested with *Aedes* and *Culex* together, a frequency one-fifth of that expected from the frequencies of each genus separately. In the urban locality, mixed infestations of the two genera were found in 5.5% of cases, one-third of that expected from random association. *Aedes* was associated with *Anopheles gambiae* in 0.3% of the positive containers in the rural villages, about one-half of that expected from random association.

Seasonal changes in prevalence

In the rural villages the proportion of containers that held water began to increase in February with the onset of the rainy season, and declined in November one month after the end of the rainy season (Fig. 1). In urban Ogui, the percentage remained fairly constant, with a slight fall in January 1975 followed by a slight rise in April. The results obtained by the conventional method of larval survey (Table 2) show that, in Ogui, the container index for *A. aegypti* rose from a dry-season average of 0.7 to a wet-season average of 3.7. At the same time, the house index increased from 4.7 to 19.5, while the Breteau index rose from 4.7 to 28.6.

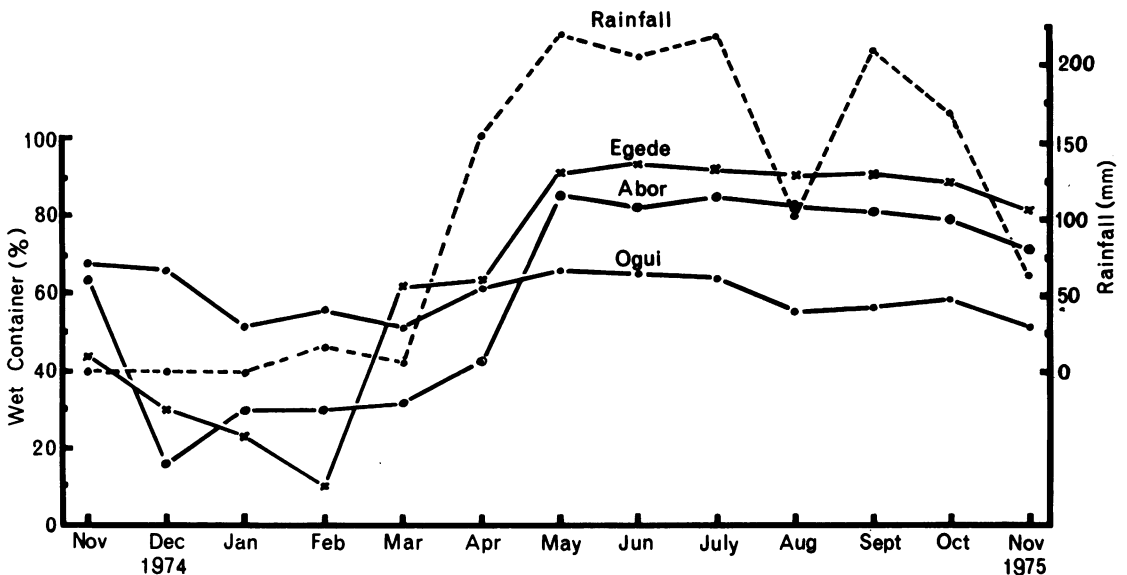


Fig. 1. Percentage of containers that held water in relation to monthly rainfall in the three study areas.

Table 2. Mean *A. aegypti* larval indices, determined by conventional larval survey, for five dry months and seven wet months

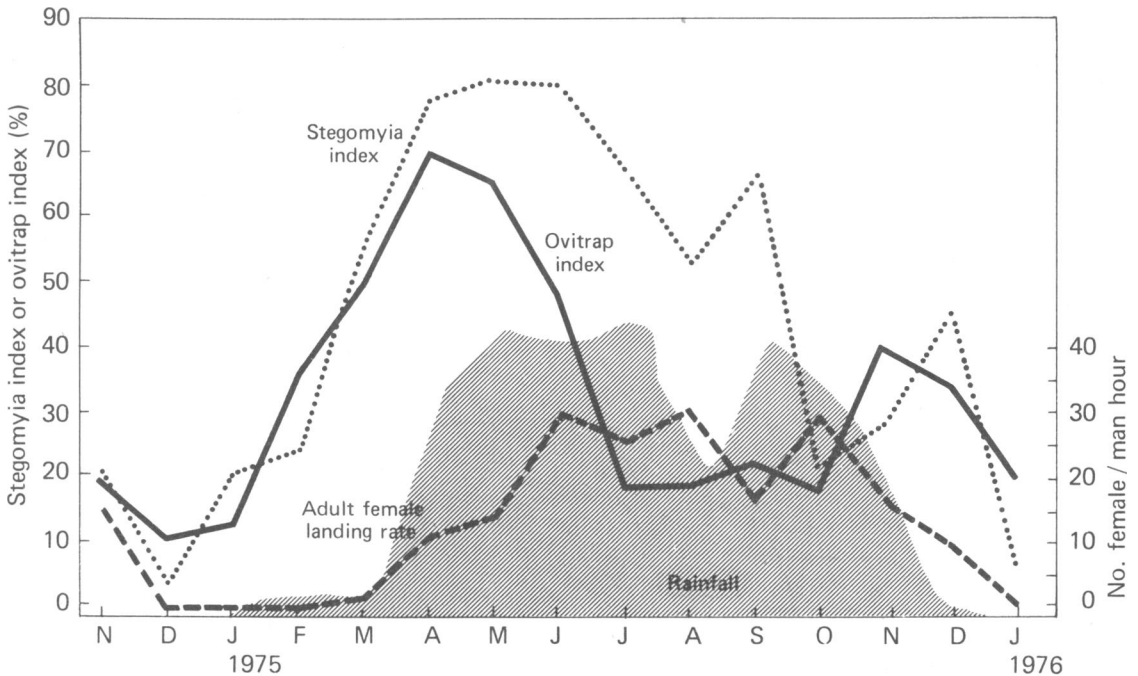
Area	Season ^a	Wet containers %	Container index	House index	Breteau index	Stegomyia index ^b
Ogui	dry	56.8	0.7	4.7	4.7	1.5
	wet	61.9	3.7	19.5	28.6	9.4
Egede	dry	31.3	1.4	7.3	11.3	29.8
	wet	89.3	2.9	41.9	76.2	202.5
Abor	dry	28.4	3.7	18.7	23.3	25.0
	wet	79.9	2.8	39.1	53.3	57.1

^a Total rainfall of 56 mm for five dry months (November–March 1975), compared with 1350 mm for seven wet months (April–October 1975), each with over 50 mm rainfall.

^b No. of positive containers per 1000 human population.

In the two rural villages, where all three indices were quite high even in the dry season, the average Breteau index in the wet season was over 50, with little change in the container index. Only in Ogui in the dry season did the Breteau index fall below 5. During the seven months with more than 50 mm of rainfall, the mean number of containers positive for *A. aegypti* per 1000 human population (Stegomyia index) was nearly 10 in

Ogui, 203 in Egede, and 57 in Abor. When the Stegomyia index was plotted against the ovitrap index (number of ovitrap paddles positive for *A. aegypti* (I)), a statistically significant correlation was found between them ($r = 0.675$, $P < 0.01$). Seasonal increase in both indices usually occurred two months before that in morning biting/landing rate of *A. aegypti* (Fig. 2).



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Fig. 2. Comparison of variations in Stegomyia index, ovitrap index, and morning landing rate of *A. aegypti* in Abor village, between November 1974 and January 1976.

Table 3. Comparison of *A. aegypti* indices determined by two sampling methods (based on geometric mean of monthly figures obtained between November 1974 and November 1975)

Indices		Locality		
		Ogui	Egede	Abor
No. of positive containers	Conventional method	79	179	155
	Single-larva-per-container method	79	175	151
	Percentage difference	0	2.3	2.6
House index	Conventional method	8.8	16.5	24.2
	Single-larva-per-container-method	8.8	16.3	23.2
	Percentage difference	0	1.4	4.1
Breteau index	Conventional method	11.1	25.4	31.9
	Single-larva-per-container method	11.1	25.0	31.2
	Percentage difference	0	1.6	2.3
Container index	Conventional method	1.5	1.8	2.7
	Single-larva-per-container method	1.5	1.6	2.6
	Percentage difference	0	1.8	2.7

Comparison of sampling methods

To compare the single-larva-per-container method with the conventional method of sampling, the geometric means of the number of positive containers infested with *A. aegypti* were calculated from the 13 monthly figures obtained with each method. In Ogui, the result for the single-larva method was the same as that determined by the conventional method (Table 3). For the two villages, however, the single-larva method gave average values that were slightly lower than those given by the conventional method, resulting in container indices that were about 2% lower. This difference was roughly equivalent to the frequency with which *Aedes* larvae were accompanied by other species. A similar small difference was found by Service (9) in north-west Nigeria. The house index and Breteau index were also slightly lower when calculated using figures obtained by the single-larva method. It therefore appears that the fraction of *Aedes* occurring in mixed infestations does not markedly reduce the larval indices found by the single-larva method.

Interrelationship of indices

To determine the relationship between the three different indices for *A. aegypti*, and their fit to the single WHO density figure (12), the 13 monthly figures for the container, house, and Breteau indices were plotted against each other (Fig. 3). Data for Egede and Ogui are plotted, since these places had, respectively, the highest and the lowest number of containers per compound. The slopes of the regression lines for Abor were always between those for Ogui and Egede. The correlation coefficients for all nine regressions from the three study sites were statistically significant.

On each of the plots, the range of index values corresponding to the single WHO density figure is also shown. In the third graph, it can be seen that the relationship between the house index and Breteau index fits that on which the WHO density figure was based. However, in both the plots involving the container index, the regression lines did not correspond with the relationship demonstrated by the WHO density figures, the divergence being greatest for Egede, which had the highest number of containers per compound. The numerical relationship between container index and Breteau index on which the WHO density figures were originally based^c was derived from simultaneous assessments of both indices in Asia and Africa, in localities where the average number of containers per compound was much lower than the 30 in Egede. In north-west Nigeria, with averages of 2-9 containers per house, Service (9) found that the container, house, and Breteau indices all gave similar WHO density figures.

Effect of sample size

Table 4 shows the effect of the number of containers sampled on the three larval indices, and is based on nine monthly assessments during the wet season from March to November. As expected, the house index and Breteau index increased as the number of containers per compound increased. On average, the house index rose by 12.5 and the Breteau index by 35 for each increase of 10 containers per compound sampled. The container index remained relatively constant despite the fact that the proportion of outdoor containers that were wet fluctuated between 50% in March and 95% in September. The

^c BROWN, A. W. A. See footnote b, page 108.

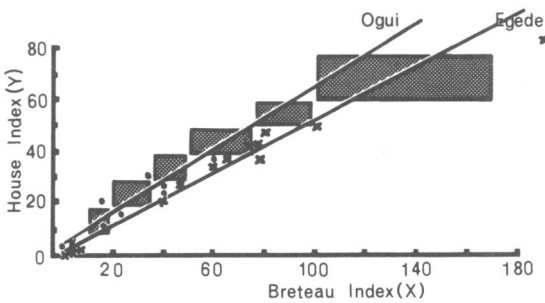
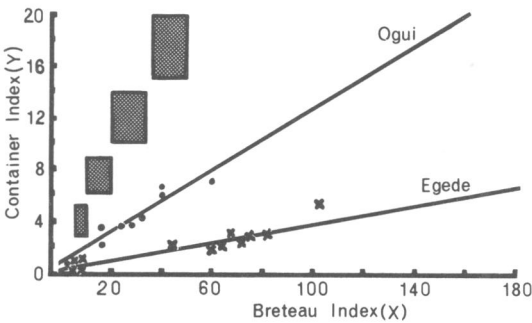
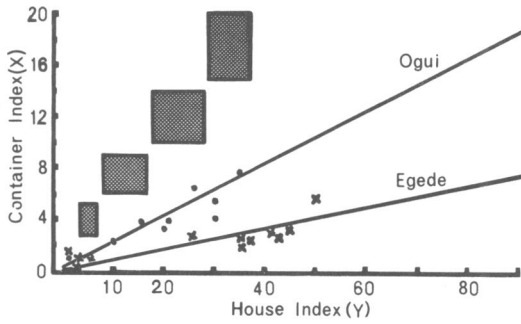


Fig. 3. Interrelationships of the three larval indices and WHO density figures for *A. aegypti*. Shaded areas indicate values of WHO index.

variation in the proportion of wet containers in each of the six groups was less than 2%, indicating that the selection of the containers had been satisfactorily random.

The expected values of the Breteau index and house index were calculated for the different groups of pots, using $1 - q^n$ for the house index, and $(1 - q) \times n$ for the Breteau index, where q = probability of a container being negative and n = number of containers in the

Table 4. Observed and expected values of indices of *A. aegypti* in relation to the number of water pots sampled

No. of pots per house (n)	Container index (p)	House index		Breteau index	
		Observed	Expected ^a	Observed	Expected ^b
5	5.7	17.5	25.4	21.9	28.5
10	5.2	27.2	41.4	40.8	52.0
15	5.2	35.6	55.1	62.2	78.0
20	4.9	40.6	63.3	77.5	98.0
25	4.8	46.1	70.8	96.9	120.0
35	4.4	56.1	79.3	126.1	154.0

^a Calculated as $(1 - q^n) \times 100$; $q = 1 - (p/100)$

^b Calculated as $p \times n$

house (Table 4). The comparison shows that the observed values are consistently lower than the expected ones, and the difference becomes greater as the number of pots per house increases.

DISCUSSION

In contrast with the situation in South-East Asia (11) and East Africa (8), a considerable variety of mosquito species was found infesting domestic water containers in this part of West Africa. Nevertheless, *Aedes* spp. other than *A. aegypti* were infrequent, and *Culex* spp., though abundant, were seldom in company with *Aedes*. Thus *A. aegypti* seldom occurred in mixed infestations, and this explains our finding that the single-larva method of survey yielded container indices similar to those obtained by the conventional method of larval survey. A similar finding has been made by Service (9) in north-west Nigeria. The use of the single-larva method would speed up larval surveys, and would be particularly valuable when a quick response is needed, e.g., in a yellow fever epidemic. It would also allow a greater number of villages to be surveyed, even in remote areas, in a given time.

An accurate measurement of larval density would require counting and identification of all the *Aedes* larvae in all the positive containers. This would be impractical (7) and surveys based on counting the number of containers positive for *Aedes* larvae have gained acceptance, the results being expressed as the container, house, or Breteau index. A table of figures has been published (13), based on results from Thailand and the United Republic of Tanzania, which relates the container index for *A. aegypti* to its absolute density in terms of larvae per hectare. However, it can only indicate that every unit increment in

the container index corresponds to population increments of between 100 and 1000 larvae per hectare. Meanwhile, the indices already in use must serve as measures of larval density.

A characteristic of the situation in south-east Nigeria which contrasts with that in other areas of *A. aegypti*'s distribution is the multiplicity of containers in the compounds surrounding the houses. Thus the production of *Aedes* can be relatively high even when the container index is low. From the point of view of potential health hazard to the population, the important parameter is the absolute number of positive containers, and not the percentage. The house index is also inadequate as an indicator of risk of disease transmission, since it does not take into account the number of positive containers in a positive house or compound. The Breteau index does reflect the number of positive containers, and indeed Chan et al. (6) in Singapore found this index to be more closely related to the real larval density than the other two indices. This index, originally called an "indice stegomyien" by Breteau (2), relates the number of positive containers to the number of houses. However, in our opinion, it is more important to relate the number of positive containers to the human population rather than to their dwellings, and so we have calculated a "Stegomyia index" as the number of positive containers per 1000 persons.

In Enugu and the adjacent Udi Hills, the yellow fever vectors breeding in domestic water containers were almost exclusively *A. aegypti*. The vector species *A. luteocephalus* occurred in 2% of the urban and 0.3% of the rural container infestations. Among other known vectors, *A. africanus* was very occasionally found in the rural areas, while *A. vittatus* was not found in this part of south-east Nigeria, in contrast with its frequent presence in water containers in north-west Nigeria (9). The average Breteau index for *A. aegypti* in the rural villages in the wet season

exceeded 50, a level which had previously been determined by Chambon et al. (5), during the yellow fever epidemic in Senegal in 1965, to be the threshold for a high risk of transmission by this species. Examination of the monthly results revealed that in the rural communities the index fell below this high-risk level only in December and January. In the Ogui quarter of Enugu city, the average wet-season Breteau index was below 30, and the dry-season average was below 5, a level where transmission of the virus was unlikely (5). The forest relict environment in which the rural villages were located apparently favoured the persistence of adult *Aedes*, and their breeding in natural niches, well into the dry season.

Taking all three indices into account, the WHO density figures for *A. aegypti* in the wet season were category 3 in Ogui and category 5 in the villages. If the container index is ignored, because of the high number of containers per compound, the density figures are 4 for Ogui and 6 for the villages. Larval density figures in this range were also found by Service (9) at the end of the wet season in north-west Nigeria, where the compounds did not usually have a large number of containers. The high-risk threshold for disease transmission has been established by WHO (12) as density figure 5. According to larval surveys, villages in density category 6 can expect an adult biting rate of about 3 per man-hour,^d while 2 per man-hour was considered to be the threshold of man-vector contact above which there is a significant risk of yellow fever transmission (12). In the two villages in the Udi Hills, the average wet-season biting rate by *Aedes* (*Stegomyia*) vectors has been found by Bang et al. (1) to be 9.7 per man-evening, equivalent to 2.4 per man-hour, *A. aegypti* contributing 0.2 per man-hour, *A. luteocephalus* 0.4 per man-hour, and *A. africanus* 1.8 per man-hour.

^d For original data, see BROWN, A. W. A., footnote b, page 108.

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RÉSUMÉ

PRÉVALENCE DES LARVES DE VECTEURS POTENTIELS DE LA FIÈVRE JAUNE DANS LES RÉCIPIENTS D'EAU À USAGE DOMESTIQUE DANS LE SUD-EST DU NIGÉRIA

Les variations saisonnières de prévalence des moustiques *Aedes* (*Stegomyia*) qui se développent dans les récipients

d'eau au voisinage des habitations ont été évaluées dans un quartier urbain d'Enugu, Nigéria, et dans deux villages

situés parmi des restes de forêts dans les collines d'Udi voisines. Un grand nombre de récipients en terre, dont la plupart contenaient de l'eau à la saison humide, se trouvaient dans les enclos entourant les habitations. Pendant 13 mois consécutifs, la présence ou l'absence de larves d'*Aedes* dans ces récipients a fait l'objet d'une détermination mensuelle. L'indice Breteau moyen (nombre de récipients contenant des larves, pour 100 habitations) évalué pour *A. aegypti* durant les sept mois de saison humide était de 53 dans l'un des villages et de 76 dans l'autre, ce qui dénotait un risque élevé de transmission de la fièvre jaune; les moyennes en saison sèche étaient de 11 et 23. Dans le quartier urbain, la moyenne en saison humide était de 29 et

en saison sèche de 4,7, niveau auquel la transmission est improbable. On a parfois trouvé dans les récipients *A. luteocephalus* dans les localités tant urbaines que rurales, et des larves d'*A. africanus* ont été découvertes dans l'un des villages. S'il était courant de trouver des larves de *Culex*, les infestations mixtes à *Aedes* et *Culex* étaient si rares que la méthode simplifiée d'échantillonnage d'*Aedes*, dite méthode «de la larve unique», a donné des résultats similaires à ceux de la méthode classique. La multiplicité des récipients autour des habitations rend donc inadéquat l'emploi de l'indice Breteau comme mesure de densité larvaire dans cette région du Nigéria.

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