

Ecology and Epidemiology of Dengue Viruses
in Din Daeng District, Bangkok.

Principal Investigators : Douglas M. Watts, Ph.D.
Bruce A. Harrison, MAJ, MSC
David E. Johnson, MAJ, MC
Terry A. Klein, CPT, MSC

Associate Investigators : Donald S. Burke, MAJ, MC
Ananda Nisalak, M.D.
Michael C. Callahan, SFC

Assistant Investigators : Panor Srisongkram, B.Sc.
Ming Choohong
Sirinipa Srilikit, R.N.
Sajee Pinnoi, R.N.
Sukree Tumrongranchani, R.N.
Panee Srangsomwong, R.N.
Nongnuj Maneechai
Chummong Noigamol
Vichit Phunkitchar
Inkam Inlao
Prajim Boonyakanist
Rampa Rattanarithikul
Kol Mongkolpanya

OBJECTIVES

1. To determine the seasonal incidence of apparent and inapparent dengue virus infections in Bangkok adults and children.
2. To establish the duration and magnitude of the antibody response to primary and secondary arbovirus infection, including dengue virus type 1, 2, 3, 4 and Chikungunya virus.
3. To assess experimentally the ability of wild *Ae. aegypti* to serve as a vector of dengue viruses on a seasonal basis.
4. To determine the population density of the wild *Ae. aegypti* population on a seasonal basis and seasonal availability and the utilization of artificial containers by this species for oviposition.

BACKGROUND : Epidemiological and ecological investigations have shown that dengue viruses are endemic in Bangkok and that the primary vector and vertebrate host are *Aedes aegypti* and man, respectively (1, 2). Apparent human infections occur throughout the year; however, a marked increase in the incidence of illness has been observed during the rainy season (June-September). Age-specific attack rate data have placed children 15 years of age or less at greatest risk (2). Early findings indicated that the variation in the incidence of infection was related to the population density of *Ae. aegypti* (3).

More recent data suggest that the magnitude of change in absolute population size of *Ae. aegypti* in Bangkok was not large enough to explain the seasonal fluctuations in the incidence of apparent dengue virus infection (4). The latter investigation was extended to consider the longevity and blood feeding patterns of *aegypti*. Data did not show seasonal variation in 24 hour survival; however, biting rates appeared to vary as indicated by a marked decrease during the cool season of the year (5). According to the authors, the decrease in biting rates and a possible increase in the length of the extrinsic incubation period of the vector may cause the decrease in the incidence of dengue virus infections in man during this part of the year.

Estimates of *Ae. aegypti* population density employing human biting and landing counts, sweep net, aspiration-vacuum sweep of resting adults, and dipping collections for larvae and pupae are biased by a number of individual human traits dependent on the collectors (6). Other collecting techniques, developed to avoid these problems include the pyrethrin knockdown, oviposition traps and the one larvae per container techniques. The former test is biased by space, timing, and sealing problems, and a reluctance of the collectors to work in the presence of pyrethrin aerosol spray. The latter two techniques are better tools, but, they cannot be employed alone for population density estimates.

METHODS

Study area : The study area for this investigation was a section of the Din Daeng area of Bangkok. The area is circumscribed by Prachasongkro Road on the north, Soi Charasongkhro on the west, Din Daeng I Road on the east and Klong Sam Sen on the south. Included in the area are 20 four-floor apartment buildings, many two-three storey shop-houses, approximately 4 acres of confluent single storey slum dwellings and a few single or two storey residential homes. The total human population of the study area was between 13,000 to 14,000.

Census of the population and mapping of the area : One hundred families of the Din Daeng study area that had a child attending Phibunprachasan School were randomly selected for the study. Each family was interviewed regarding address, income level, occupation, family size, ages, general health, and cultural and behavioral practices possibly related to disease recognition. Interviews of the families and the mosquito habitat surveys were conducted concurrently and on a seasonal basis. The entire area was mapped in regard to layout of housing, streets and other permanent landmarks,

Dengue virus infection of the human population : The seasonal incidence of dengue and Chikungunya infections was based on seroconversion rates determined by hemagglutination inhibition tests (7). Blood was obtained from family members before and after each season of the year. Overt dengue virus infections were determined by bi-weekly visits to the residence of each family in the study population. Classification of apparent and inapparent infection was based on previously defined criteria (8, 9).

Immature mosquito habitat surveillance : The availability and utilization of *Ae. aegypti* immature habitats were determined 4 times (each season of the year) between April 1978 - February 1979. In March 1979 the frequency of

surveys for immature habitats was increased to every 6 weeks to gain more information for interpreting *aegypti* population trends. During the first 2 surveys in 1978, very little natural vegetation and no natural containers were found in the vicinity of the study residences. Accordingly, the immature habitat surveys concentrated on artificial containers. Residences of 100 families were surveyed both indoors and outdoors to provide an estimate of the number of potential and positive larval *aegypti* habitats. Families leaving the present study for any of a variety of reasons were replaced with randomly selected families in the same study area. All artificial containers within the boundary of each family residence were surveyed. Plastic containers, pails, washpans and other containers emptied on a daily basis were recorded, but not included since they did not usually provide a source for immature mosquitoes and therefore rarely contributed to the *aegypti* population.

Artificial containers defined as "inside" were determined on the basis of a roof or roof-like structure that precluded rain water from entering the container. Containers defined as "outside" were determined on the basis of no roof or roof-like structure, or if a roof was present, it did not preclude rain water from entering the container. Lids or covers may occur on containers in both categories. Each artificial container with water was searched thoroughly for pupae and 1st to 4th stage larvae using a flashlight and a 4 ounce suction syringe. Water from containers that could not be visually searched, e.g., vases, was poured into a white pan, immature mosquitoes removed, and water then replaced in the original container. All immature mosquitoes were brought to the laboratory, identified, separated on the basis of larvae or pupae, and then, either pooled for virus isolation or discarded.

Standardized water containers (ong jar) were purchased and one was placed in the residence of each of the 100 families selected for study. These jars will serve to provide estimates of the population density of *Ae. aegypti* obtained through concurrent employment of different, complementary sampling techniques. Each sampling device is being designed to focus on a certain life stage, behavioral factor, and/or physiological state of the mosquito population. The sampling methods (traps) described below are being designed to eliminate the bias of the human traits listed above and will be employed, provided preliminary tests show them to be reliable and effective sampling devices.

A floating larval trap will be used to determine the population density of *Ae. aegypti* larvae and pupae. These traps are used on a rotating schedule throughout the 100 family units and collections are made during one 24 hour period each week.

Emergence cone traps that fit over the mouth of water jars will be used to determine adult emergence patterns and densities during the year.

An estimate of the oviposition rate of *Ae. aegypti* will be determined seasonally by estimating the number of gravid females. This will be determined by capturing gravid females in a specially designed trap that captures the female, but does not allow her to reach a substrate for oviposition.

Additional sampling for estimating the adult density will be attempted by using a suction type trap. This trap will be designed to sample active flying adults, or by attracting them to a resting site.

RESULTS

Laboratory studies : Several aspects of *Ae. aegypti* biology and behavior, and their relationships to dengue virus transmission were reported previously (10). These studies involved: (A) the extrinsic incubation periods of dengue viruses 1-4 in *aegypti*; (B) transovarial transmission of dengue viruses by *aegypti*; and (C) the developmental periods and longevity of immatures and adults of *aegypti* under different conditions. Two of these studies have expanded during this study period. Consequently, they have been treated as separate studies and are reported elsewhere in this report, i.e., transovarial transmission (11) and developmental and longevity studies (12). Due to technical problems there was little progress with the extrinsic incubation studies.

Vector competence studies: Experimental studies designed to determine the susceptibility of wild *aegypti* to infection with dengue viruses during the different seasons of the year, were discontinued due to the lack of a reliable method for infecting and demonstrating virus transmission by mosquitoes. In addition, studies could not be conducted to determine the susceptibility of selected strains of *aegypti* to dengue virus infection. The latter strains have been characterized according to their isoenzyme profiles as reported elsewhere in this report (13). A hanging drop technique was employed as described previously (10), and is somewhat similar to the method previously reported (14) to be effective for infecting *Ae. aegypti* with dengue viruses and for demonstrating virus transmission.

Although it was possible to infect low numbers of *Ae. aegypti* by allowing them to feed on a hanging drop of a dengue virus-blood suspension, the feeding rate was usually less than 10 per cent. Of the mosquitoes that became infected, none transmitted detectable levels of virus to a hanging drop of blood after an incubation period of up to 35 days. Attempts to employ the hanging drop technique by others have resulted in limited success (15). These studies will be continued provided an effective technique can be developed. Meanwhile, eggs of *aegypti*, from the different seasons of the year will be stored, as well as eggs of the different colony strains of *aegypti*, to provide mosquitoes for testing. More recent findings (D.M. Watts, personal communication) indicate that rhesus monkeys infected with dengue viruses may prove to be a suitable host for infecting *aegypti*.

Adult and Larval Traps: The development of various larval and adult traps continued during this period and resulted in one larval trap and 3 adult traps which consistently yielded many adults or larvae in laboratory tests. An emergence trap consisting of a clear plastic cone (35 cm tall) painted black inside, a funnel and a small clear plastic container, was designed to fit snugly over the top of the standardized (28 liter) water jars placed in each study residence. The plastic cone has a 3-4 cm hole at the tip which fits inside a funnel with a 4 cm long spout. The funnel spout is then inserted through a hole drilled through the bottom of a clear circular 13 cm wide and 8 cm deep plastic container (with lid) that traps the mosquitoes. This trap was placed over the study jars containing 4th stage *aegypti* larvae for 3 time periods in laboratory tests, 24, 48 and 72 hrs. During laboratory tests, the traps set for 48 hrs consistently yielded a large percentage of the hatching

mosquitoes and kept a majority of the adults alive. The emergence trap became part of the routine field surveillance program in the Din Daeng Study area in January, 1978 (see Field Studies).

Another adult trap was designed to simulate an oviposition site and trap the females that entered. This trap consisted of a large, round clear plastic container (16.5 cm diam. x 26.5 cm tall) painted black on the outside, with a large hole cut in the lid into which a funnel fits snugly (top of funnel flush with the lid) and with the spout pointing downward inside the container. Inside this container a small clear plastic container (trap cage) is attached to the funnel spout by inserting the spout through a small hole in the container lid. The bottom of the small inside container was at least 5-7 cm above the bottom of the large container so that about 4 cm of water (from *aegypti* positive water jars) could be placed in the large container. In addition, the funnel had several small screened panels in the cone portion so that water vapor from inside the trap could be detected in the funnel. Female *aegypti* attracted to the combination of black container-water vapor would fly down into the trap via the funnel spout and become trapped in the small container below, because they could not find the small funnel spout entrance again.

In laboratory tests with known numbers of mosquitoes, this trap worked very well, and was found equally effective for both sexes, gravid females and non-gravid females of *Ae. aegypti* and *Culex quinquefasciatus*. Based on the laboratory tests the trap was presumed to be generally attractive as a resting site, and became part of the field studies in Din Daeng in April 1978. After a one month period in the field study residences, however, this trap did not capture a sufficient number of specimens to warrant its continued use. Probable reasons for this failure outside the laboratory are: (A) low adult *aegypti* numbers in the study residences (see emergence trap data); (B) competition with numerous other artificial water containers in the study residences (see the container indices for each type of residence); and (C) the failure of the black jar to be attractive in the poorly lit, dark interiors of most of the study residences.

The third trap consisted of a motorized suction trap (CDC) run on 4 flashlight batteries. The light source and lid of this standard trap were removed and replaced with a glossy black, square wooden box (8 cm tall x 19 cm on each side) with a hole in the bottom, which fit snugly over the top of the plastic frame of the CDC trap. Along each of the 4 sides of the black box there was a single narrow (10-15 mm) air vent through which air was drawn by the trap motor. The trap was designed so that adult *aegypti* would be attracted to the shiny black box and sucked through the lateral vents down into the standard net bag below the trap. In consecutive 24 hr (1200 to 1200) laboratory tests this trap consistently captured large numbers of adults, particularly male *aegypti*. After many laboratory trials, this trap was integrated into the field studies in Din Daeng in April 1979. However, as with the above resting trap, the number of adults captured during a one month field trial was too small to warrant its continued use. Beside low adult *aegypti* numbers in the study residences and the failure of the black box to attract adults in the dimly lit interiors, other problems were encountered. The large size of the trap in the very small (one room) residences caused some discomfort to the

residents. In addition, finding a means to suspend the trap caused difficulties in some residences. This trap uniformly caught a fair number of *Culex quinquefasciatus* in the study residences; however, a standard CDC trap with light, probably would have caught many more adults of this latter species.

During the last reporting period a very efficient floating larval trap was designed and tested in the laboratory (10). This trap will float in any water container holding 10 cm or more water and is ideally suited for trapping in the ceramic water jars, cement bath tanks and metal drums which are used to store water in the tropics. The trap keeps the larvae/pupae alive during a 24 hr trapping period, is very stable, quickly returning to an upright position if turned over, and floats randomly on the water surface, eliminating the human bias of collecting only in concentrations of larvae.

During this reporting period laboratory tests with the larval trap were concluded and this trap was integrated into the Din Daeng field studies in January 1979 (see Field Studies).

FIELD STUDIES : Dengue virus infection of the human population. The prevalence of antibody to dengue virus in those families resident in high-rise apartments (flats) differed from those families living in shop houses or the slums. There was no observed difference in the last 2 and they have been combined for purposes of this report. Figures 1 and 2 present the 1978 pre-dengue season prevalence of antibody against dengue viruses and Chikungunya virus, respectively. While the prevalence of anti-Chikungunya antibody varied only slightly by dwelling type, housing appeared to have a noticeable effect on dengue antibody in children. Regardless of housing, dengue antibody prevalence approached 100% by age 20.

Figures 3 and 4 present the incidence of dengue virus infection in Din Daeng residents. Figure 3 represents only those individuals who did not have evidence of antibody prior to the start of the period under examination. Twenty-six primary seroconversions were documented during the early rainy season (June-September 1978) and 8 were observed during the late rainy-early cool season (September 1978 - January 1979). The incidence between housing types was not statistically significant, but the difference in antibody acquisition between the two periods was noticeable ($X^2_y = 5.98$, $p < 0.02$). Figure 4 presents the data for the same periods in individuals who had previously exhibited an antibody titer to one or more dengue types. In contrast to primary infections, the secondary type response was not different between the 2 periods. Shop and slum housing accounted for significantly more secondary infections in the late rainy season than did the flats ($X^2_y = 15.96$, $p < 0.005$). This was the only subgroup in which housing type was associated with a difference in the incidence of dengue infection.

Artificial container surveillance. Investigations of the seasonal availability and utilization of artificial containers by *Ae. aegypti* in the Din Daeng study area were initiated in April 1978 (10) and have continued to the present. The percentage and frequency of potential artificial containers with water are shown in Figure 5. The percentage of containers with water inside residences ranged from 69.5 to 84.6%, while the containers with water outside residences ranged from 94.6 to 100.00%, except for the initial survey when it was 61.2%.

However, during each survey the frequency of inside containers with water was 6 times greater than the frequency of outside containers with water. The low number of outside artificial containers with water at the study residences was probably due to: (A) the limited space at most residences, leaving little room for more than a dwelling; and (B) the structural design of the high-rise flats. Outside containers were mostly confined to the slum residences, with a few located in the shop residences. High-rise flats (approximately 50% of the total residences surveyed) because of vertical design, did not have outside space for outside containers.

The frequency and percentage of artificial containers positive for *aegypti* are shown in Figure 6. The percentage of artificial containers positive for *aegypti* was higher outside than inside residences during all trapping periods. However, since slum residences contribute the majority of outside containers, this phenomenon appears to be based on residence-type rather than outside versus inside containers.

Although inconclusive at this time, the data indicate that the mosquito population density, in terms of number of positive containers, is influenced by both mean monthly temperature and precipitation (Figure 7). It appears that changes in the percentage and frequency of artificial containers positive for *Ae. aegypti* are reflections of changes in the mean monthly temperatures, i.e., with increases in the mean monthly temperature there are observed increases in the number of positive artificial containers, and conversely, decreases in the mean monthly temperature result in lower numbers of positive artificial containers.

Increases in the percentage and frequency of positive containers are also associated with periods of increased precipitation, i.e., during the rainy season. It appears that the mosquito population density, as determined by frequency of positive artificial containers, is first influenced by increases in the mean monthly temperature, beginning with the hot dry season. With the end of the hot dry season and the beginning of the rainy season, the frequency of positive artificial containers continued to increase. However, near the end of the rainy season and the beginning of the cool dry season, a decrease in the frequency of positive containers was observed. The observed changes in per cent of positive containers outside residences are more noticeable than for containers inside residences, whereas changes in frequency are much smaller. This difference is primarily due to the limited numbers of artificial containers outside the study residences. Outside containers, with the exception of ong jars, are also dependent upon rain as a water source. However most of the outside containers were ong jars and were filled by rain and/or tap water.

The frequency and percentage of different types of containers positive for *aegypti* inside and outside study residences are summarized in Table 1. Three major types of artificial containers (ong jars, bath and foot basins, and ant-traps) were found to be the primary habitats for immature *argypti*. A fourth type, flower vase/base, although relatively abundant, was positive infrequently (< 3.3 per cent).

Containers classified as "others", although included, were not considered to be a major contributing factor primarily because of their small numbers and their very low positive rate. Slum residences are almost totally responsible for all "other" containers. Most slum residences have a small area adjacent to the house where small children play or water containers are stored, and occasional "other" containers are stored or discarded.

Comparing all of the containers, a higher percentage of ant traps were positive, ranging from 20.8 to 52.5 per cent for all trapping periods. Ong jars and bath and foot basins were next, ranging from 13.1 to 39.1 and 9.8 to 32.5 per cent respectively (Figure 8). Although bath and foot basins and ant traps, when present, were frequently observed to be positive, they were limited in number and, in the case of bath and foot basins, were almost entirely restricted to high-rise flats and shop residences. For this reason, it became necessary to obtain a "positive container index" for the different types of residences, i.e., the number of positive containers per residence. This provides a more reliable index in evaluating seasonal population changes and an estimation of the type of containers most responsible for changes in the seasonal abundance of *aegypti* (Tables 2, 3, 4 and 5). Overall, ong jars are more frequently observed positive than all other containers combined (0.31 to 1.94). Ant traps and bath and foot basins follow, ranging from 0.12 to 0.33 and 0.05 to 0.13, respectively (Figure 9).

The high-rise flat residences, in general, have a very low positive container index when compared to the other type residences. The containers most frequently positive were ong jars, with bath and foot tanks, ant traps and flower vases/bases positive only occasionally (Figure 10). Of the three types of study residences, slum residences had the highest positive container index (Figure 11). Ong jars accounted for the majority of positive containers, while the remainder of positive containers were primarily ant traps. Bath and foot tanks were infrequent in the slum residences and infrequently positive. Shop residences, unlike slum residences, do not have one type of container accounting for the majority of all positive containers (Figure 12). Also shop residences were the only type residence where there was a significant number of positive flower vases/bases.

Changes in the mean number of different types of containers infested with *Ae. aegypti* were similar, in most cases, to the total seasonal fluctuation in positive inside and outside containers. Ong jars, because of their greater numbers and common use in all three residence types, appear to provide a better index of mosquito population density changes in relation to frequency of positive containers. However, a more in depth evaluation of mosquito population changes can be provided for shop residences, when ong jars, ant traps and bath and foot basins are included in a surveillance program,

Per cent seasonal changes in the study residences having different types of containers positive for *aegypti* are summarized in Table 6. The percentage of all study residences positive (Figure 13) is directly associated with changes in the mean number of positive containers. The percentage of high-rise flat residences positive for *aegypti* varied from 4.2 to 26.5 per cent for all surveys and was very small when compared with shop and slum residences. The percentage of shop and slum residences positive for *aegypti* were similar,

varying from 45.0 to 88.2 and 51.6 to 84.4 per cent respectively. Although the total percentage of positive shop and slum residences were nearly the same for each trapping period, the type of container which contributed to the residences being positive varied. Ong jars were almost totally responsible for slum residences being positive, with bath tanks accounting for less than 7 per cent and ant traps for less than 17 per cent during any one trapping period. In general, if a slum residence had positive bath tanks and/or ant traps, it also had at least one positive ong jar. Although ong jars play a major role in shop residences being positive, they were responsible for more than 50 per cent of shop residences being positive on only 3 occasions. Positive bath tanks were especially prevalent (15.0-52.6 per cent) in shop houses, while ant traps and flower vases were positive between 20-35 per cent of the time.

Figures 14, 15, 16 and 17 illustrate the uneven distribution of the different types of positive containers in the 3 types of residences. Slum houses were more frequently positive for ong jars than either shop or high-rise flat residences (Figure 14), while shop residences were more frequently positive for bath and foot tanks and flower vases (Figure 15 and 16). There was no obvious difference in the number of positive ant traps between the slum and shop residences (Figure 17). Apparently, the primary difference between high-rise flats and shop residences was their respective accessibility to the mosquitoes. Both shops and flat residences had similar types and quantities of artificial containers. However, shops were open, and on ground level, allowing easy access for adult *aegypti*, while the 4 story high-rise flats usually had screened windows and occasionally screened doors, and had the entry way at least one story above ground level.

Mosquito population density surveillance. Originally, mosquito density estimates were going to be based on the results of 4 trapping programs, i.e., larval trap, emergence trap, black resting trap and the black box-suction trap. These traps were all developed and laboratory tested, with good to excellent results, by early 1979. However, as discussed in the laboratory studies section, only a few adult *aegypti* were collected by the black resting trap and the black box-suction trap after a one month test in the study residences. Accordingly, surveillance with these 2 traps was discontinued. The larval and emergence traps have been monitoring *aegypti* population densities since January 1979. Since routine container surveillance determines the prevalence of positive jars, the larval and emergence traps were set only in/on ong (water) jars that contained larvae or pupae. By this means data were accumulated on changes in the abundance of *aegypti* in known positive containers. The data for the larval and emergence traps are presented separately below.

Emergence trap surveillance. A uniform sized water container was selected to provide a standardized immature habitat for estimating the population densities of both emerging adults and immatures of *aegypti*, and to facilitate the use of uniform sized traps, particularly the larger emergence trap. Accordingly, a standardized ceramic water jar (AFRIMS ong jar: 40 cm x 80 cm, approximately 28 liter volume) was placed in each residence of the 100 families involved in the study. New randomly selected families replacing departing families were also provided with an AFRIMS jar. Broken or lost AFRIMS jars were replaced with new jars.

The schedule for setting the emergence traps each trapping period always preceded the larval trapping period. Using this schedule, the highly efficient larval traps did not have a detrimental effect on the adult *aegypti* emergence rates.

Four trapping periods have been completed and the results tabulated (Table 7). Emergence traps were set for approximately 48 hrs, and only on *aegypti* positive AFRIMS jars. The actual trapping time ranged from 43.8 to 53.8 hrs, with a mean of 47.9 hrs. The prevalence of *aegypti* positive AFRIMS jars during any one survey was consistently low. Accordingly, only a few emergence traps were set each trapping period and the results are inconclusive. However, the data indicate a trend of increased emergence associated with rising mean monthly temperatures, which also continues to increase after the onset of the rainy season. Male:female ratios of adult *aegypti* captured in the emergence traps ranged from 1:0.7 to 1:1.4 for the different trapping periods. The average male:female ratio for the 4 trapping periods was 1:1. Data are currently insufficient for indicating seasonal male:female ratios.

Larval trap surveillance. Surveillance of immature mosquitoes by a larval trap was initiated January 1979, and 5 trapping periods have been completed. This trap was designed to provide an unbiased estimate of immature mosquito population densities, and to assess possible seasonal differences in *aegypti* abundance. Ong (water) jars, including AFRIMS ong jars, were trapped in all 3 categories of residences, i.e., high-rise flats, shops and slums. Ong jars were selected as the source of surveillance primarily because of: (A) a large numbers of ong jars in the study area; (B) previous surveys demonstrated a large percentage of the jars were positive; (C) their abundance in all of the types of study residences surveyed; and (D) physical limiting factors of the trap design, primarily its size (13 cm diam. x 13 cm tall). All ong jars with water were surveyed in the study residences. Larval traps were set only in ong jars with observed immature mosquitoes, and removed approximately 24 hrs later.

The actual range and mean of total time for completed larval trap surveys were 17.9 to 28.8 hrs and 23.6 hrs, respectively. Immature mosquitoes captured in the larval traps were separated as pupae or larvae, identified and recorded. Larval trap surveillance will continue into 1980.

The positive jar index (Figure 18) more accurately reflects the actual *aegypti* positive jar rate per type of residence than the percentage of positive ong jars per type of residence. This is due to a disparity in the average number of ong jars per residence, per type of residence (high-rise flat 2.3, shop 2.7, slum 4.9). The tabulated data also indicate that the percentage of ong jars positive for *aegypti* increases following the onset of increased amounts of precipitation (Figures 7 and 19). Although all 3 types of residences demonstrated an increase in positive ong jars following the onset of periods of increased precipitation, the slum residences had the greatest increase, while the high-rise flat residences had the smallest.

The average number of larvae, pupae and combined total¹ of immature mosquitoes per sampled² ong jar for the study residences are summarized in Table 8. More trapping is required to get a better perspective of the cyclic nature of immature *aegypti* populations in relation to mean monthly temperature and precipitation. However, the data accrued to date, indicate that the average number of mosquitoes per sampled ong jar increases with the beginning of the hot season and even further after the onset of rainy periods. The frequency of immature *aegypti* trapped per sampled ong jar ranged from 0 to 512, with less than 5 per cent of the jars negative. The high-rise flats had the highest average number of immature *aegypti* per positive ong jar (positive ong jar index), while the slums had the lowest number. However, the reverse is true when comparing the total number of ong jars with water, i.e., the slums had the highest average numbers of immature *aegypti* per ong jar (ong jar index) while the high-rise flats had the lowest. The relationships of mean monthly temperature and precipitation to the number of pupae as compared to the numbers of larvae are currently inconclusive.

Similar mosquito density-meteorological relationships although less conclusive, were also demonstrated for the seasonal frequency and per cent of different types of study residences with ong jars infested with immature *aegypti*. The slums demonstrated the highest percentage of positive residences (76.5-83.3 per cent), while the high-rise flats had the lowest percentage positive residences (4.2-16.7 per cent). The shop residences were intermediate and ranged from 41.2-50.0 per cent positive for all 5 trapping periods. The percentage of all residences positive for immature *aegypti* demonstrated very little change and only increased following heavy periods of precipitation (Figures 7 and 20).

These studies are continuing.

REFERENCES :

1. Johnson, K.M., S.B. Halstead and S.N. Cohan, 1967. Hemorrhagic Fevers of Southeast Asia and South America: A Comparative Appraisal. Prog. Med. Virol. 9: 105-58.
2. Halstead, S.B., J.E. Scanlon, P. Umpaivat and S. Udomsakdi, 1969, Dengue and Chikungunya Virus Infection in Man in Thailand, 1962-1964, IV, Epidemiological Studies in the Bangkok Metropolitan Area. Am. J. Trop. Med. Hyg. 18: 997-1021.
3. Scanlon, J.E. 1966. Bangkok Haemorrhagic Fever Investigations: The 1962-63 Mosquito Collections. Bull. W.H.O. 35: 82-3.
4. Sheppard, P.M., W.W. Macdonald, W.J. Tonn and B. Grab, 1969, The Dynamics of an Adult Population of *Aedes aegypti* in Relation to Dengue Haemorrhagic Fever in Bangkok. J. Anim. Ecol. 38: 661-702.

¹ Larvae and pupae not separated for first 2 trapping phases.

² Occasionally we were not allowed trapping access to positive jars.

5. Yasuno, M. and R.J. Tonn. 1970. A Study of Biting Habits of *Aedes aegypti* in Bangkok, Thailand. Bull. W.H.O. 43: 319-25.
6. Service, M.W. 1976. Mosquito Ecology: Field Sampling Methods. Halstead Press, John Wiley and Sons, New York. 583 pp.
7. Clarke, D.H. and J. Casals. 1958. Techniques for Hemagglutination and Hemagglutination Inhibition with Arthropod-borne Viruses. Am. J. Trop. Med. Hyg. 7: 561-73.
8. Winter, P.E., T.M. Yuill, S. Udomsakdi, D.J. Gould, S. Nantapanich and P.K. Russell. 1968. An Insular Outbreak of Dengue Haemorrhagic Fever. I. Epidemiological Observations. Am. J. Trop. Med. Hyg. 17: 590-9.
9. Likosky, W.H., C.H. Calisher, A.I. Michelson, R. Correa-Coronas, B.E. Henderson and R.A. Feldman. 1973. An Epidemiological Study of Dengue Type 2 in Puerto Rico 1969. Am. J. Epidemiol. 97: 264-75.
10. Watts, D.M., B.A. Harrison and D.E. Johnson. 1978. Ecology and Epidemiology Studies of Dengue Viruses in Din Daeng, Bangkok, Thailand. AFRIMS Annual Progress Report, October 1977-September 1978, pp. 66-79.

Table 8. LARVAL TRAP : Average number of immature *Aedes aegypti* per positive ong (water) jar trapped for study residences, Din Daeng, Bangkok, Thailand, (1979)

Date of Survey	Average number of <i>Ae. aegypti</i> larvae per sampled ong jar			Average number of <i>Ae. aegypti</i> pupae per sampled ong jar			Average number of <i>Ae. aegypti</i> larvae & pupae sampled ong jar		
	Inside	Outside ¹	Total	Inside	Outside ¹	Total	Inside	Outside ¹	Total
24 JAN - 15 FEB 79	-	-	-	-	-	-	33.9 (1626/48)	39.3 (785/20)	35.5 (2411/68)
5-22 MAR 79	-	-	-	-	-	-	33.9 (1932/57)	25.4 (915/36)	30.6 (2847/93)
16 APR - 4 MAY 79	35.3 (1942/55)	16.6 (466/28)	29.0 (2408/83)	1.0 (54/55)	1.1 (30/28)	1.0 (84/83)	36.3 (1996/55)	17.7 (496/28)	30.0 (2492/83)
29 MAY - 15 JUN 79	38.6 (2778/72)	40.2 (1086/27)	39.0 (3864/99)	1.5 (111/72)	2.9 (78/27)	1.9 (189/99)	40.1 (2889/72)	43.1 (1164/27)	40.9 (4053/99)
9-27 JUL 79	33.2 (2720/82)	20.2 (788/39)	29.0 (3508/121)	0.88 (72/82)	0.62 (24/39)	0.79 (96/121)	34.04 (2792/82)	20.8 (812/39)	29.8 (3604/121)

¹ Only includes slum & shop study residences.

Table 7. Emergence Trap : Seasonal variation of positive emergence traps for AFRIMS ong jar¹ for houses, Din Daeng, Bangkok, Thailand, 1979

Date of Survey	Number Jars Surveyed			Number Jars Positive for <i>A. aegypti</i> Larvae & Pupae			Number of Emergence Traps Positive			Percent Traps Positive for Positive Jars		
	Inside	Outside	Total	Inside	Outside	Total	Inside	Outside	Total	Inside	Outside	Total
22 JAN - 15 FEB 79	75	6	81	24	5	29	9	5	14	37.5	100.0	48.3
5-21 MAR 79	76	7	83	17	3	20	10	1	11	58.8	33.3	55.0
16 APR - 4 MAY 79	74	6	80	16	3	19	4	0	4	25.0	0.0	21.1
9-27 JULY 79	79	1	80	24	1	25	13	0	13	54.2	0.0	52.0

¹ One standard size ong jar (40 cm x 80 cm) was placed in each household for trapping purposes.

Table 6. Percent seasonal changes in study residences having different types of containers positive for *Aedes aegypti*, Din Daeng, Bangkok, Thailand, 1978-79

Containers	Date of Survey							
	3-18 APR 78	30 MAY - 15 JUN 78	18-28 SEPT 78	6-15 DEC 78	5-22 MAR 79	16 APR - 4 MAY 79	29 MAY - 15 JUN 79	9-27 JUL 79
Water Jar	19.0 (19/100)	29.0 (29/100)	39.0 (39/100)	30.3 (30/99)	38.4 (38/99)	36.1 (35/97)	37.5 (36/96)	45.7 (43/94)
Bath & Foot Tanks	5.0 (5/100)	13.0 (13/100)	12.0 (12/100)	7.1 (7/99)	4.0 (4/99)	4.1 (4/97)	9.4 (9/96)	10.6 (10/94)
Flower Vase & Clay Pot Base	2.0 (2/100)	3.0 (3/100)	2.0 (2/100)	2.0 (2/99)	4.0 (4/99)	2.1 (2/97)	0.0 (0/96)	1.1 (1/94)
Ant Trap	10.0 (10/100)	9.0 (9/100)	8.0 (8/100)	5.1 (5/99)	5.1 (5/99)	7.2 (7/97)	6.3 (6/96)	8.5 (8/94)
Other ¹	3.0 (3/100)	3.0 (3/100)	0.0 (0/100)	1.0 (1/99)	2.0 (2/99)	1.0 (1/97)	2.1 (2/96)	2.1 (2/96)
Total	29.0 (29/100)	43.0 (43/100)	53.0 (53/100)	38.4 (38/99)	42.4 (42/99)	42.3 (41/97)	45.8 (44/96)	53.2 (50/94)
Inside Containers	25.0 (25/100)	37.0 (37/100)	46.0 (46/100)	34.3 (34/99)	36.4 (36/99)	33.0 (32/97)	42.7 (41/96)	51.1 (48/94)
Outside Containers	8.0 (8/100)	14.0 (14/100)	15.0 (15/100)	10.1 (10/99)	17.2 (17/99)	17.5 (17/97)	13.5 (13/96)	16.0 (15/94)

¹ Plastic containers, pails, wash pans and other artificial containers emptied on a dicaly basis not included.

² Residences positive for more than one type of container or positive both inside and outside only counted once.

Table 5. Positive container index for immature *Aedes aegypti* per slum residence (outside-inside)
Din Daeng, Bangkok, Thailand (1978-79)

Containers	Date of Survey							
	3-18 APR 78	30 MAY - 15 JUN 78	18-28 SEPT 78	6-15 DEC 78	5-22 MAR 79	16 APR - 4 MAY 79	29 MAY - 15 JUN 79	9-27 JUL 78
Water Jar	0.74 (23/31)	1.34 (43/32)	1.65 (56/34)	1.21 (40/33)	2.09 (71/34)	1.97 (65/33)	2.41 (77/32)	3.13 (94/30)
Bath & Foot Tanks	0.03 (1/31)	0.06 (2/32)	0.03 (1/34)	0.0 (0/33)	0.0 (0/34)	0.0 (0/33)	0.03 (1/32)	0.03 (1/30)
Flower Vase & Clay Pot Base	0.0 (0/31)	0.0 (0/32)	0.0 (0/34)	0.0 (0/33)	0.0 (0/34)	0.0 (0/33)	0.0 (0/32)	0.0 (0/30)
Ant Trap	0.35 (11/31)	0.47 (15/32)	0.26 (9/34)	0.27 (9/33)	0.32 (11/34)	0.27 (9/33)	0.31 (10/32)	0.37 (11/30)
Other ¹	0.13 (4/31)	0.09 (3/32)	0.0 (0/34)	0.06 (2/33)	0.06 (2/34)	0.03 (1/33)	0.06 (2/32)	0.07 (2/30)
Total	1.26 (39/31)	1.97 (63/32)	1.94 (66/34)	1.55 (51/33)	2.47 (84/34)	2.27 (75/33)	2.81 (90/32)	3.60 (108/30)

¹ Plastic containers, pails, wash pans and other artificial containers emptied on a daily basis not included.

Table 4. Positive container index for immature *Aedes aegypti* per shop residence (outside-inside)
Din Daeng, Bangkok, Thailand (1978-79)

Containers	Date of Survey							
	3-18 APR 78	30 MAY - 15 JUN 78	18-28 SEPT 78	6-15 DEC 78	5-22 MAR 79	16 APR - 4 MAY 79	29 MAY - 15 JUN 79	9-27 JUL 79
Water Jar	0.15 (3/20)	0.32 (6/19)	0.47 (8/17)	0.35 (6/17)	0.82 (14/17)	0.69 (11/16)	0.94 (16/17)	1.13 (18/16)
Bath & Foot Tanks	0.15 (3/20)	0.5 (10/19)	0.53 (9/17)	0.35 (6/17)	0.18 (3/17)	0.31 (5/16)	0.59 (10/17)	0.38 (6/16)
Flower Vase & Clay Pot Base	0.2 (4/20)	0.05 (1/19)	0.12 (2/17)	0.18 (3/17)	0.11 (2/17)	0.19 (3/16)	0.06 (1/17)	0.19 (3/16)
Ant Trap	0.2 (4/20)	0.79 (15/19)	0.29 (5/17)	0.18 (3/17)	0.35 (6/17)	0.44 (7/16)	0.24 (4/17)	0.38 (6/16)
Other ¹	0.0 (0/20)	0.0 (0/19)	0.0 (0/17)	0.0 (0/17)	0.0 (0/17)	0.0 (0/16)	0.0 (0/17)	0.0 (0/16)
Total	0.7 (14/20)	1.7 (32/19)	1.41 (24/17)	1.06 (18/17)	1.47 (25/17)	1.63 (26/16)	1.82 (31/17)	2.06 (33/16)

¹ Plastic containers, pails, wash pans and other artificial containers emptied on a daily basis not included.

Table 3. Positive container index for immature *Aedes aegypti* per high-rise flat (inside), Din Daeng, Bangkok, Thailand (1978-79)

Containers	Date of Survey							
	3-18 APR 78	30 MAY - 15 JUN 78	18-28 SEPT 78	6-15 DEC 78	5-22 MAR 79	16 APR - 4 MAY 79	29 MAY - 15 JUN 79	9-27 JUL 79
Water jar	0.10 (5/49)	0.16 (8/49)	0.27 (13/49)	0.16 (8/49)	0.10 (5/48)	0.15 (7/48)	0.17 (8/47)	0.25 (12/48)
Bath & Foot Tanks	0.02 (1/49)	0.02 (1/49)	0.06 (3/49)	0.02 (1/49)	0.02 (1/48)	0.0 (0/48)	0.0 (0.47)	0.08 (4/48)
Flower Vase & Clay Pot Base	0.0 (0/49)	0.06 (3/49)	0.0 (0/49)	0.0 (0/49)	0.04 (2/48)	0.0 (0/48)	0.0 (0/47)	0.0 (0/48)
Ant Trap	0.14 (7/49)	0.06 (3/49)	0.04 (2/49)	0.0 (0/49)	0.0 (0/48)	0.0 (0/48)	0.09 (4/47)	0.04 (2/48)
Other ¹	0.0 (0/49)	0.0 (0/49)	0.0 (0/49)	0.0 (0/49)	0.0 (0/48)	0.0 (0/48)	0.0 (0/47)	0.0 (0/48)
Total	0.27 (13/49)	0.30 (15/49)	0.37 (14/49)	0.18 (9/49)	0.17 (8/48)	0.15 (7/48)	0.26 (12/47)	0.38 (18/47)

¹ Plastic containers, pails, wash pans and other artificial containers emptied on a dialy basis not included.

Table 2. Positive container index (mean) for immature *Aedes aegypti* per study residence (outside & inside), Din Daeng, Bangkok, Thailand, 1978 - 79

Containers	Date of Survey							
	3-18 APR 78	30 MAY - 15 JUN 78	18-28 SEPT 78	6-15 DEC 78	5-22 MAR 79	16 APR - 4 MAY 79	29 MAY - 15 JUN 79	9-27 JUL 79
Water jar	0.31 (31/100)	0.57 (57/100)	0.77 (77/100)	0.55 (54/99)	0.98 (97/99)	0.86 (83/97)	1.05 (101/96)	1.94 (124/94)
Bath & Foot Tanks	0.05 (5/100)	0.13 (13/100)	0.13 (13/100)	0.07 (7/99)	0.04 (4/99)	0.05 (5/97)	0.11 (11/96)	0.12 (11/94)
Flower Vase & Clay Pot Base	0.04 (4/100)	0.04 (4/100)	0.02 (2/100)	0.03 (3/99)	0.04 (4/99)	0.03 (3/97)	0.01 (1/96)	0.03 (3/94)
Ant Trap	0.22 (22/100)	0.33 (33/100)	0.16 (16/100)	0.12 (12/99)	0.17 (17/99)	0.16 (16/97)	0.19 (18/96)	0.20 (19/94)
Other ¹	0.04 (4/100)	0.03 (3/100)	0.0 (0/57)	0.02 (2/99)	0.02 (2/99)	0.01 (1/97)	0.02 (2/96)	0.02 (2/94)
Total	0.66 (66/100)	1.10 (110/100)	1.08 (108/100)	0.79 (78/99)	1.18 (117/99)	1.11 (108/97)	1.39 (133/96)	1.69 (159/94)

¹ Plastic containers, pails, wash pans and other artificial containers emptied on a daily basis not included.

TABLE 1. Frequency and percentage of inside and outside containers¹ with water positive for immature *Aedes aegypti* in study residences by surveys, Din Daeng, Bangkok, Thailand 1978-79

Containers	Date of Survey							
	3-18 APR 78	30 MAY - 15 JUN 78	18-28 SEPT 78	6-15 DEC 78	5-22 MAR 79	16 APR - 4 MAY 79	29 MAY - 15 JUN 79	9-27 JUL 79
Water jar	13.1 (31/236)	22.7 (57/251)	25.0 (77/308)	18.2 (54/297)	29.1 (97/333)	26.2 (83/317)	31.8 (101/318)	39.1 (124/317)
Baht & Foot tanks	9.8 (5/51)	32.5 (13/40)	27.7 (13/47)	14.0 (7/50)	8.9 (4/45)	10.6 (5/47)	23.4 (11/47)	22.9 (11/48)
Flower vase & clay pot base	2.7 (4/149)	3.3 (4/120)	1.4 (2/146)	2.1 (3/140)	2.4 (4/164)	2.2 (3/134)	0.64 (1/155)	1.9 (3/155)
Ant trap	28.2 (22/78)	52.4 (33/63)	20.8 (16/77)	26.1 (12/46)	34.7 (17/49)	38.1 (16/42)	50.0 (18/36)	48.7 (19/39)
Other ¹	26.7 (4/15)	9.1 (3/33)	0.0 (0/7)	3.3 (2/6)	50.0 (2/4)	100.0 (1/1)	25.0 (2/8)	66.7 (2/3)
Total	12.5 (66/529)	22.5 (110/489)	18.5 (108/585)	14.5 (78/539)	20.8 (124/595)	20.0 (108/541)	23.6 (133/564)	28.3 (159/562)

¹ Plastic containers, pails, wash pans and other containers emptied on a daily basis not included.

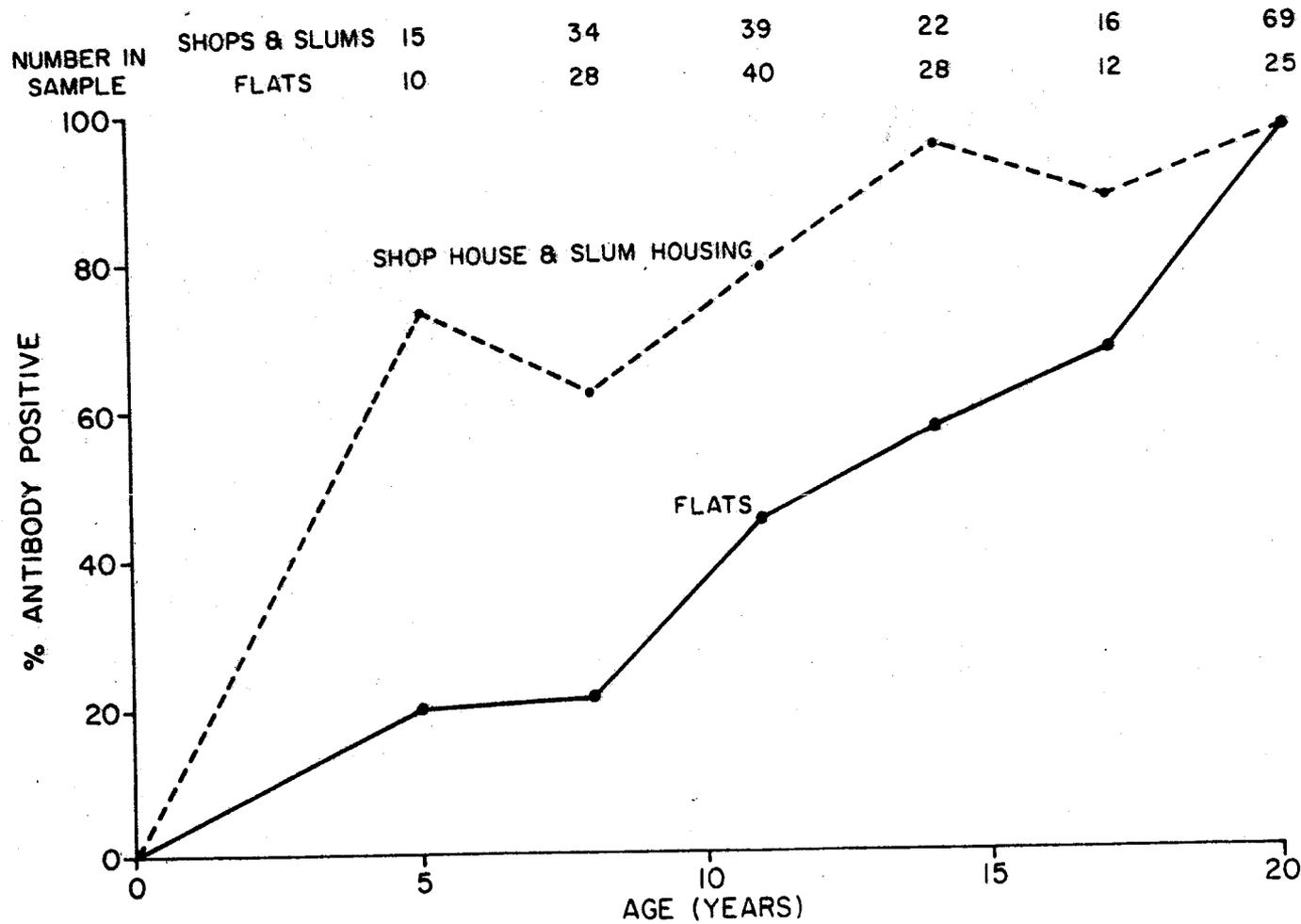


FIG. 1. PREVALENCE OF ONE OR MORE DENGUE ANTIBODY TITERS, DIN DAENG, THAILAND 1978

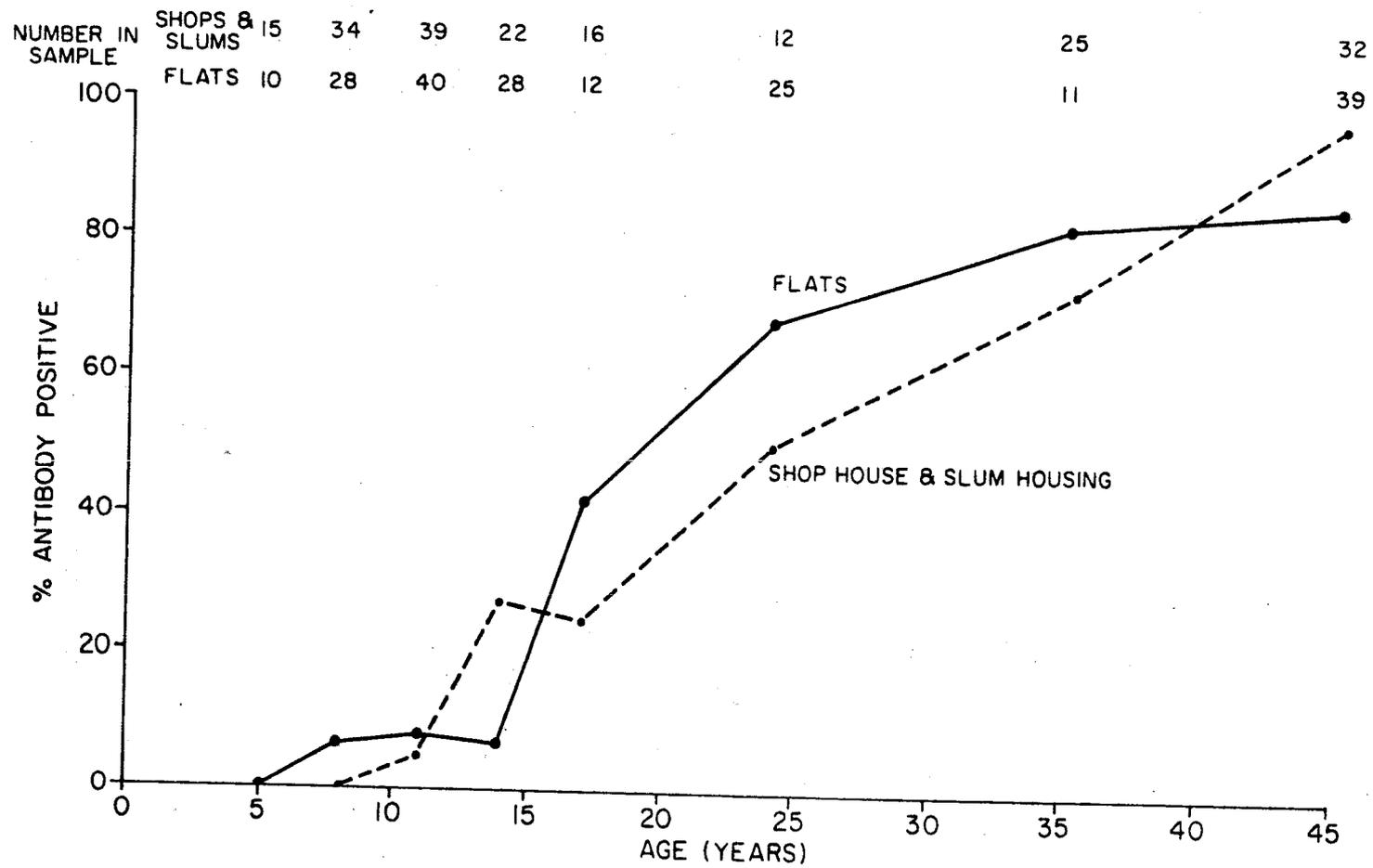


FIG. 2. PREVALENCE OF CHIKUNGUNYA VIRUS ANTIBODY, DIN DAENG, THAILAND 1978.

FIG. 3. INCIDENCE OF DENGUE ANTIBODY ACQUISITION IN PREVIOUSLY UNINFECTED INDIVIDUALS
DIN DAENG, BANGKOK THAILAND.

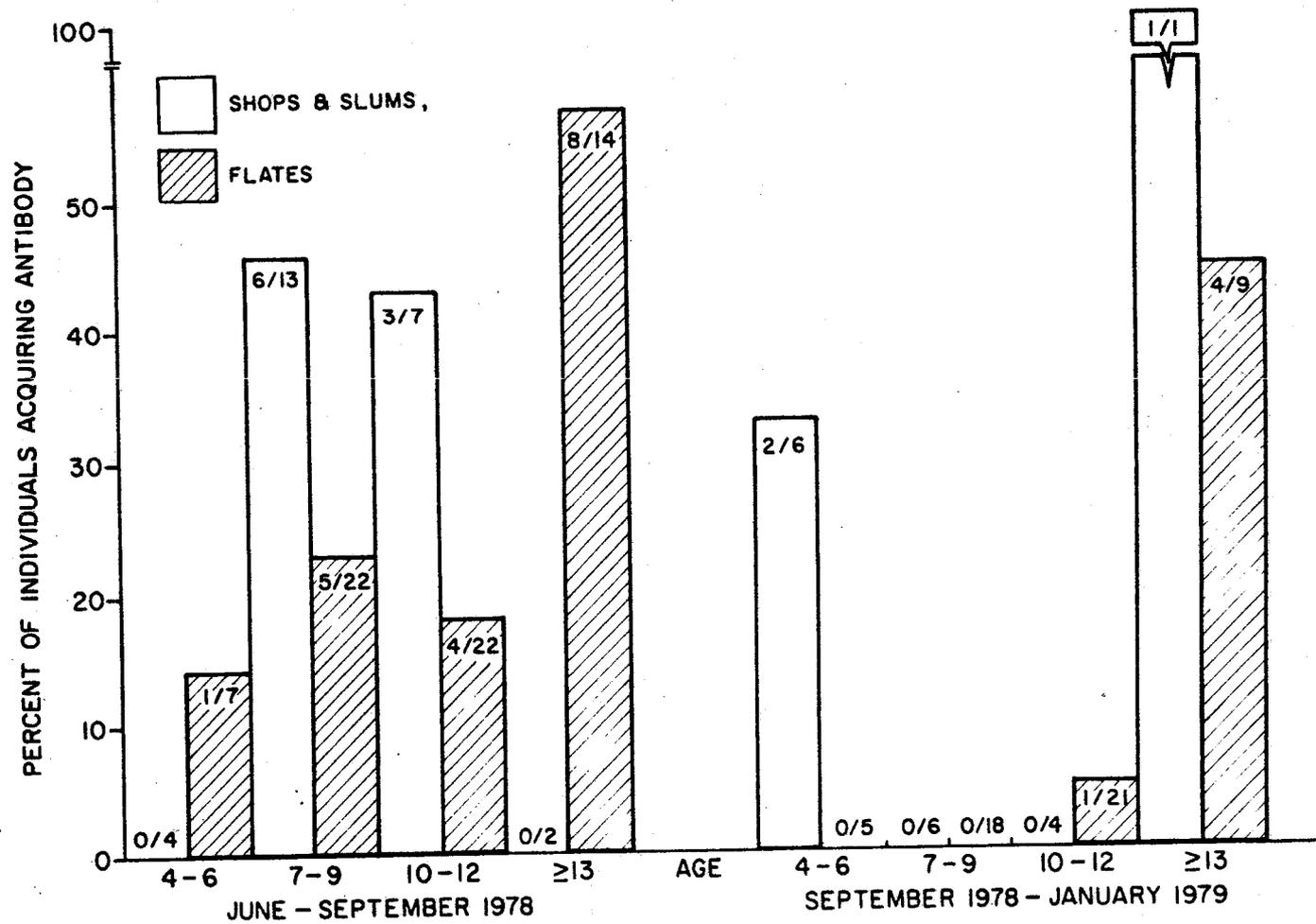
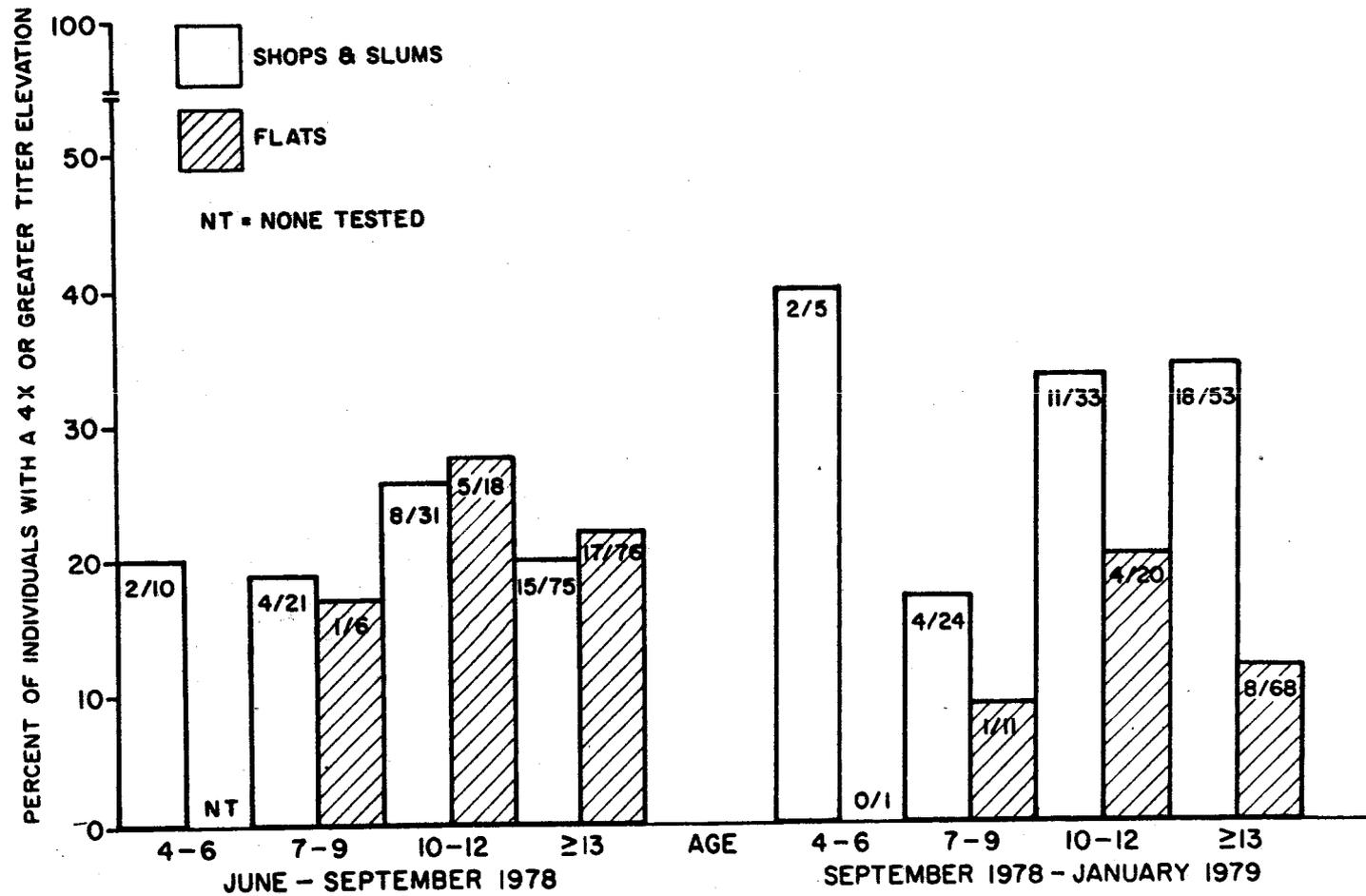


FIG. 4. INCIDENCE OF DENGUE TITER ELEVATION IN PREVIOUSLY INFECTED INDIVIDUALS
DIN DAENG, BANGKOK THAILAND.



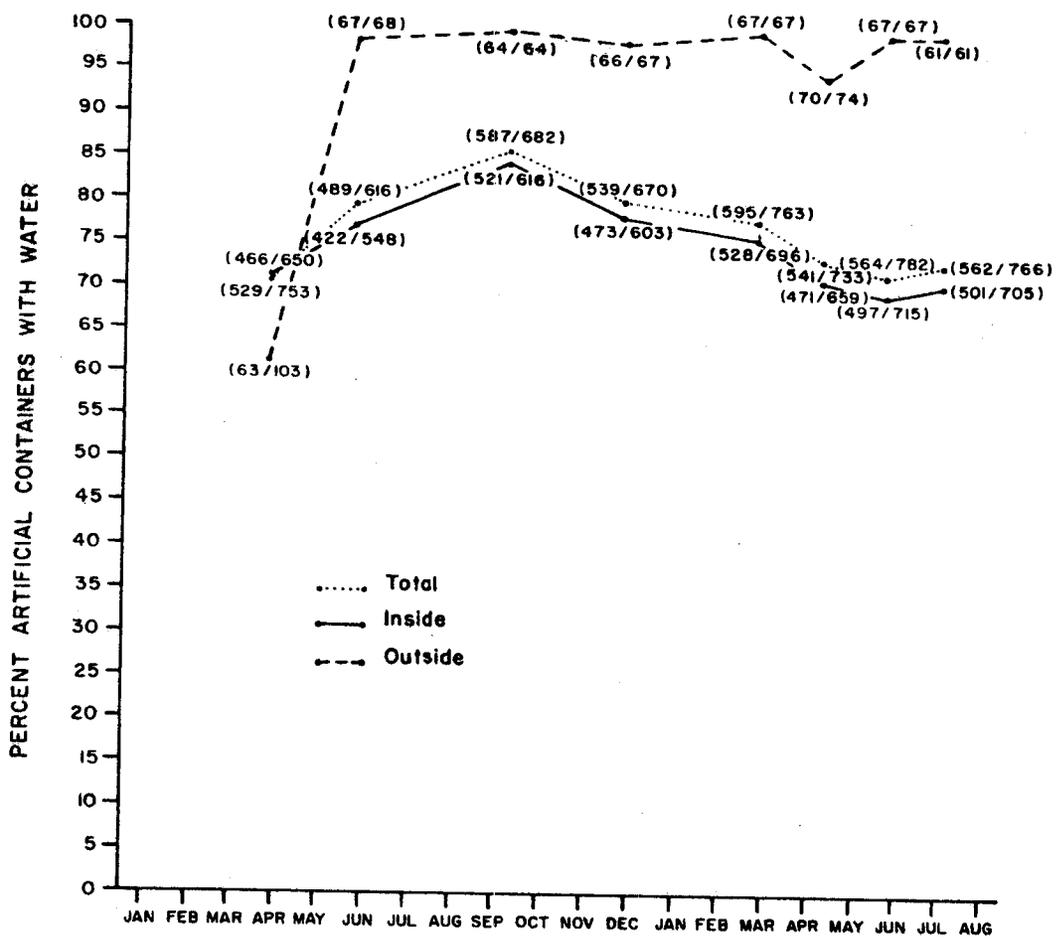


Figure 5. Percentage of artificial water containers¹ with water in study residences, Din Daeng, Bangkok, Thailand (1978-1979).

¹ Plastic containers, pails, wash pans and other artificial containers emptied on a daily basis not included.

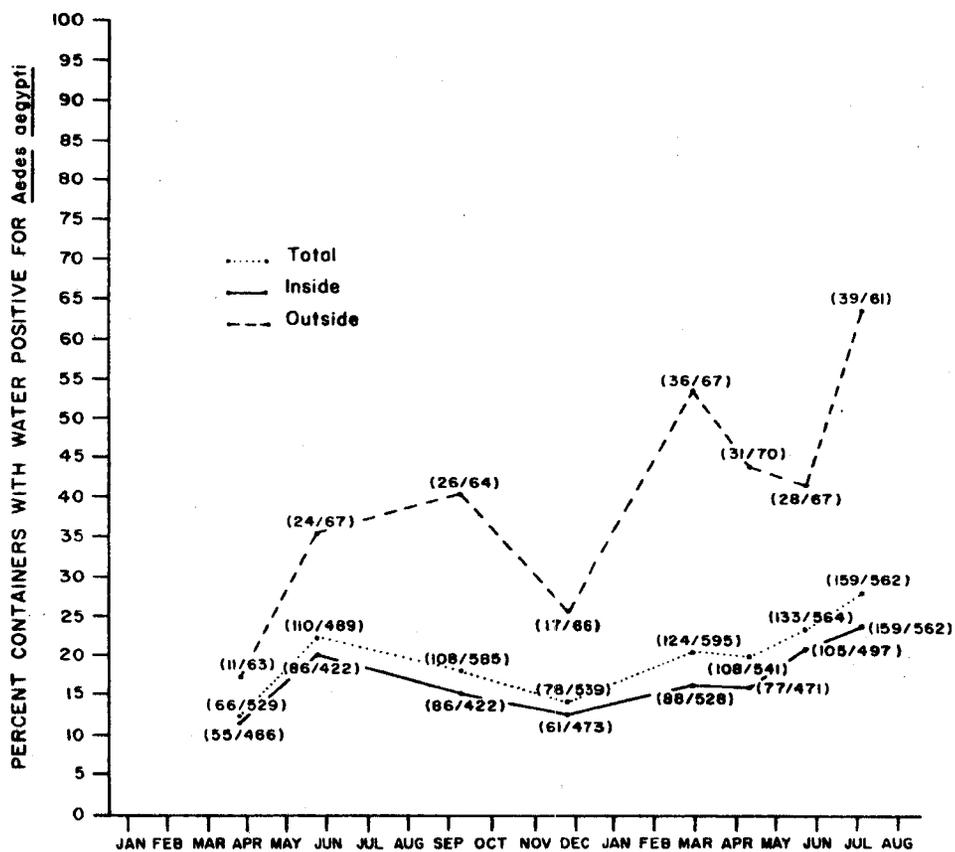


Figure 6. Percent artificial containers¹ with water positive for immature *Aedes aegypti* inside and outside residences by seasons, Din Daeng, Bangkok, Thailand (1978-1979).

¹ Plastic containers, pails, wash pans, and other artificial containers emptied on a daily basis not included.

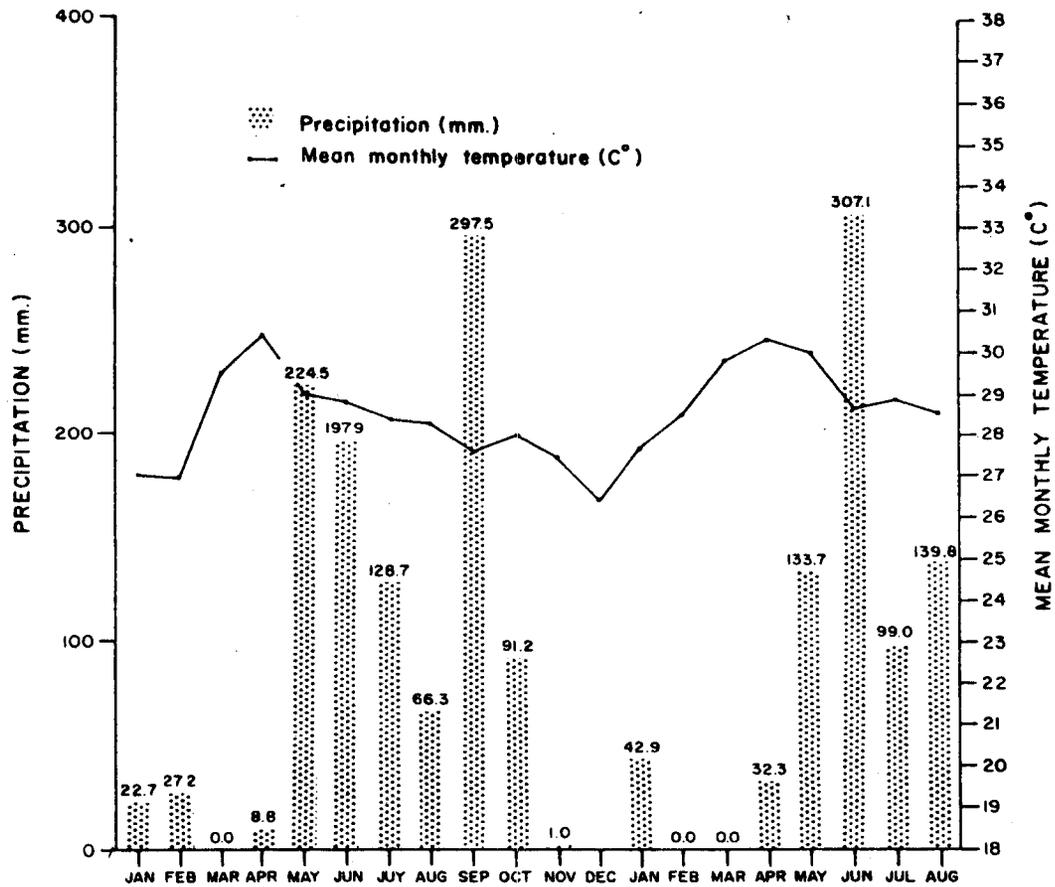


Figure 7. Precipitation¹ and mean monthly temperatures² recorded for January 1978 through August 1979.

¹ Engineering School, Meteorological Department, Ministry of Communications, Din Daeng, Bangkok, Thailand.

² Climatology Division, Meteorological Department, Ministry of Communications, Bangkok, Thailand.

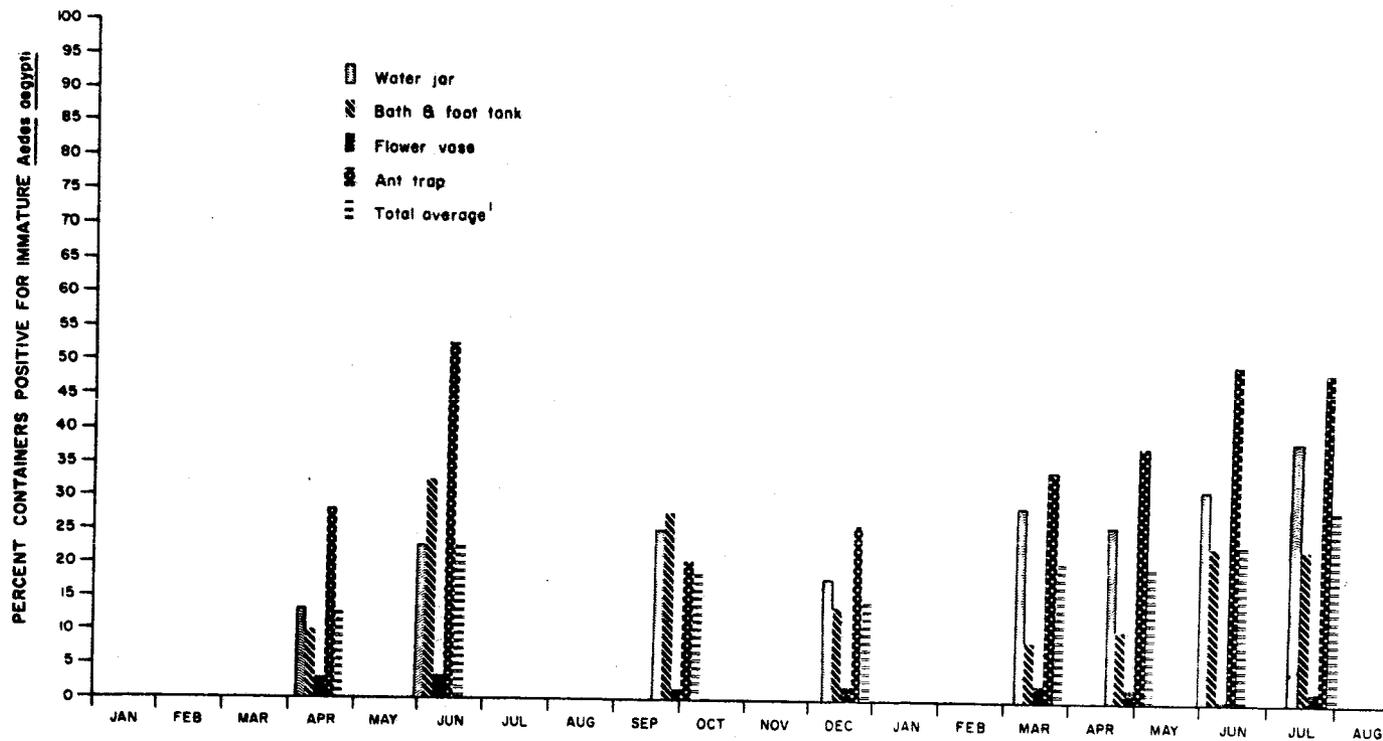


Figure 8. Percentage of different types of containers positive for immature *Aedes aegypti* inside & outside study residence, Din Daeng, Bangkok, Thailand (1978-1979).

¹Includes other containers.

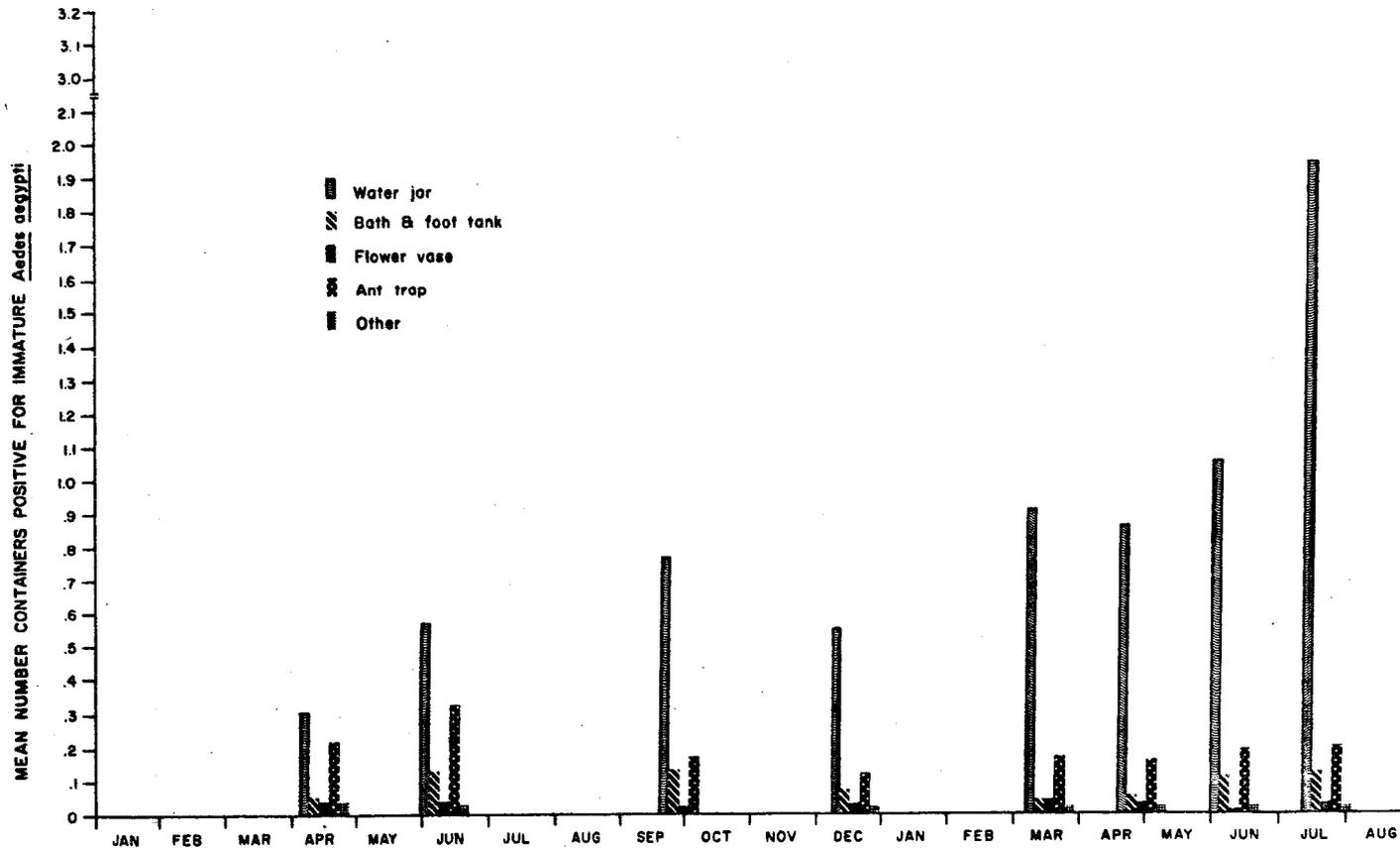


Figure 9. Mean number of containers positive for immature *Aedes aegypti* per study residence (outside & inside), Din Daeng, Bangkok, Thailand (1978-1979).

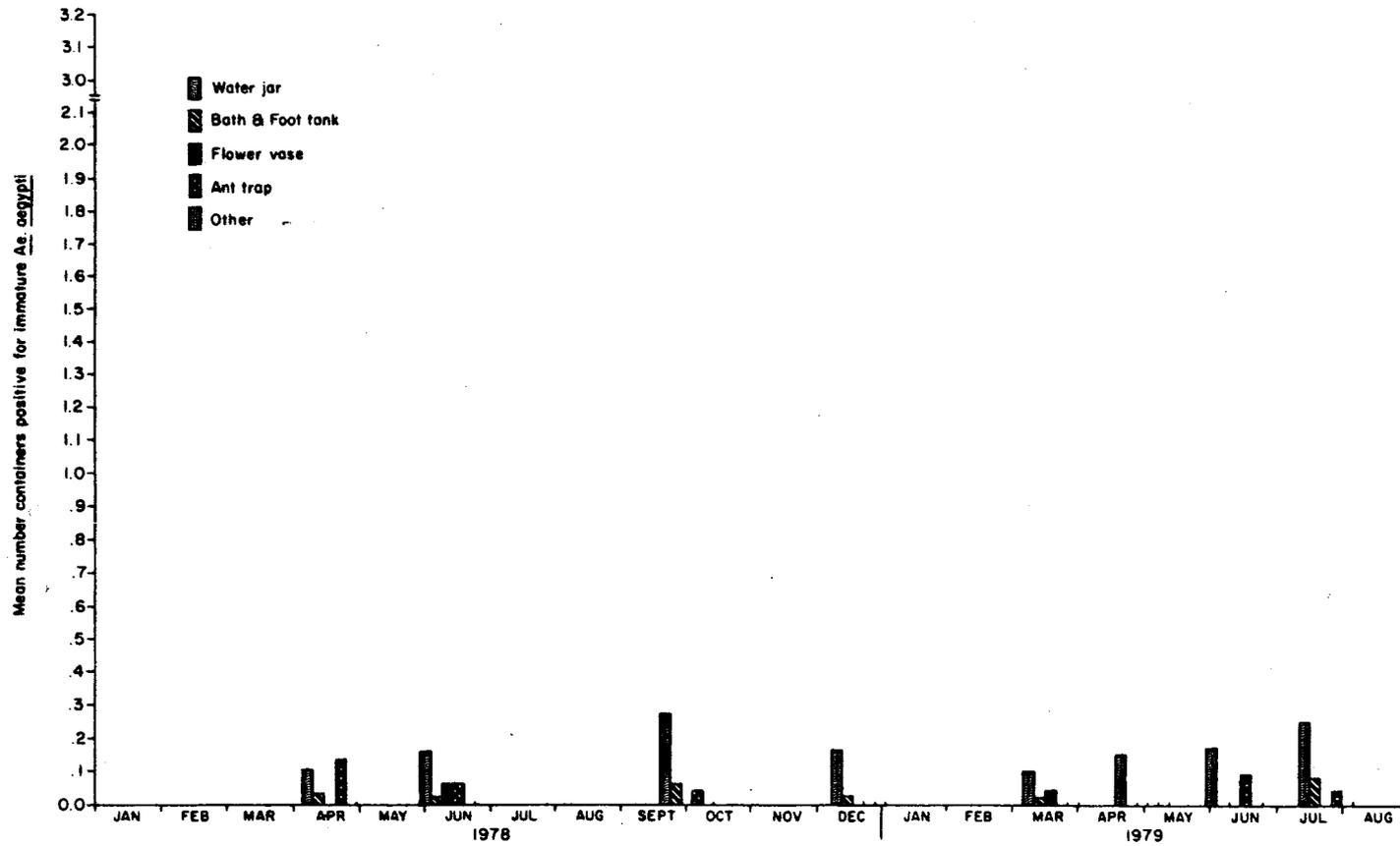


Figure 10. Mean number of containers positive for immature *Aedes aegypti* per high-rise flat (inside), Din Daeng, Bangkok, Thailand (1978 - 79)

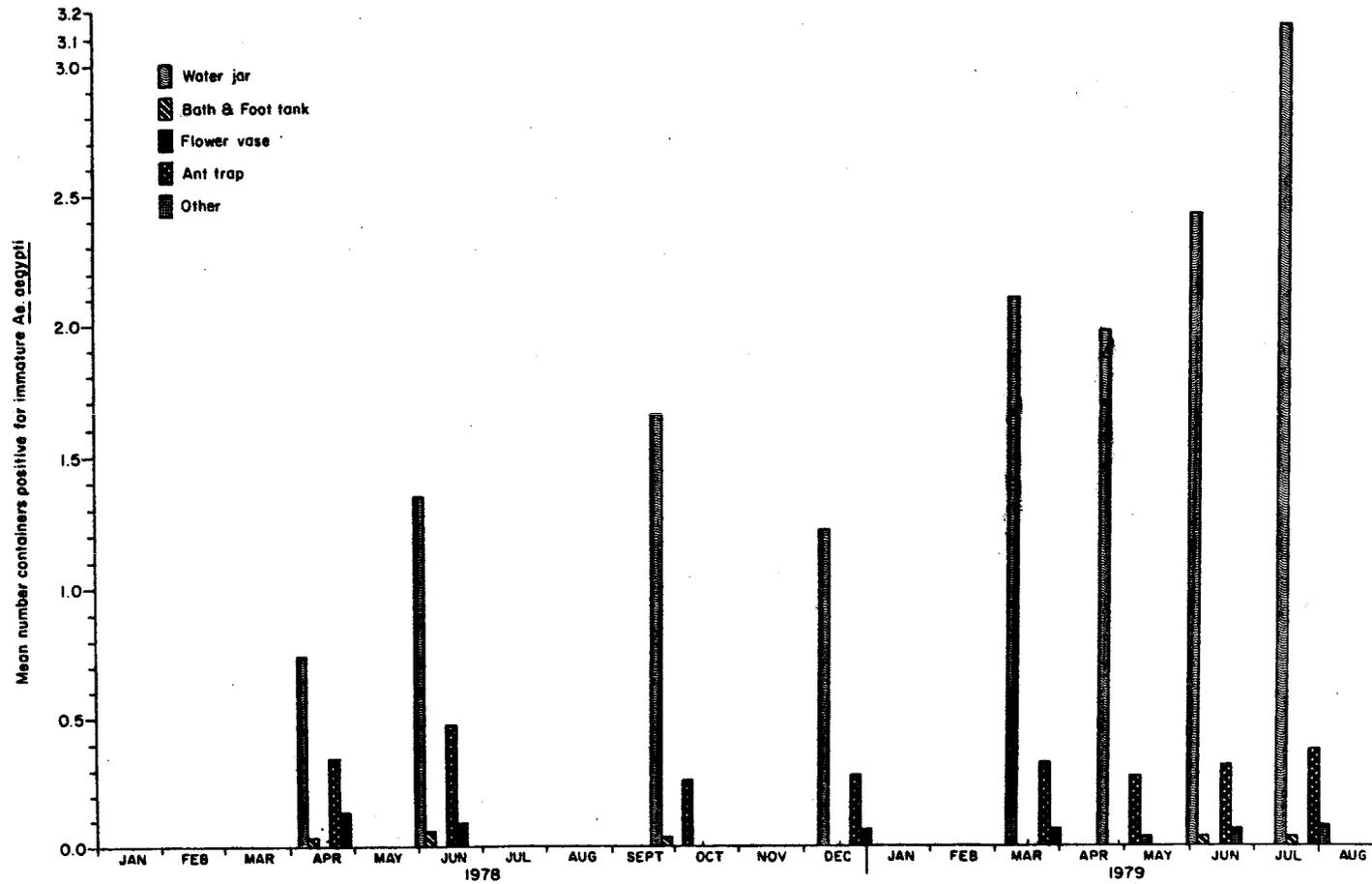


Figure II. Mean number of containers positive for immature *Aedes aegypti* per slum residence (outside & inside), Din Daeng, Bangkok, Thailand. (1978-79)

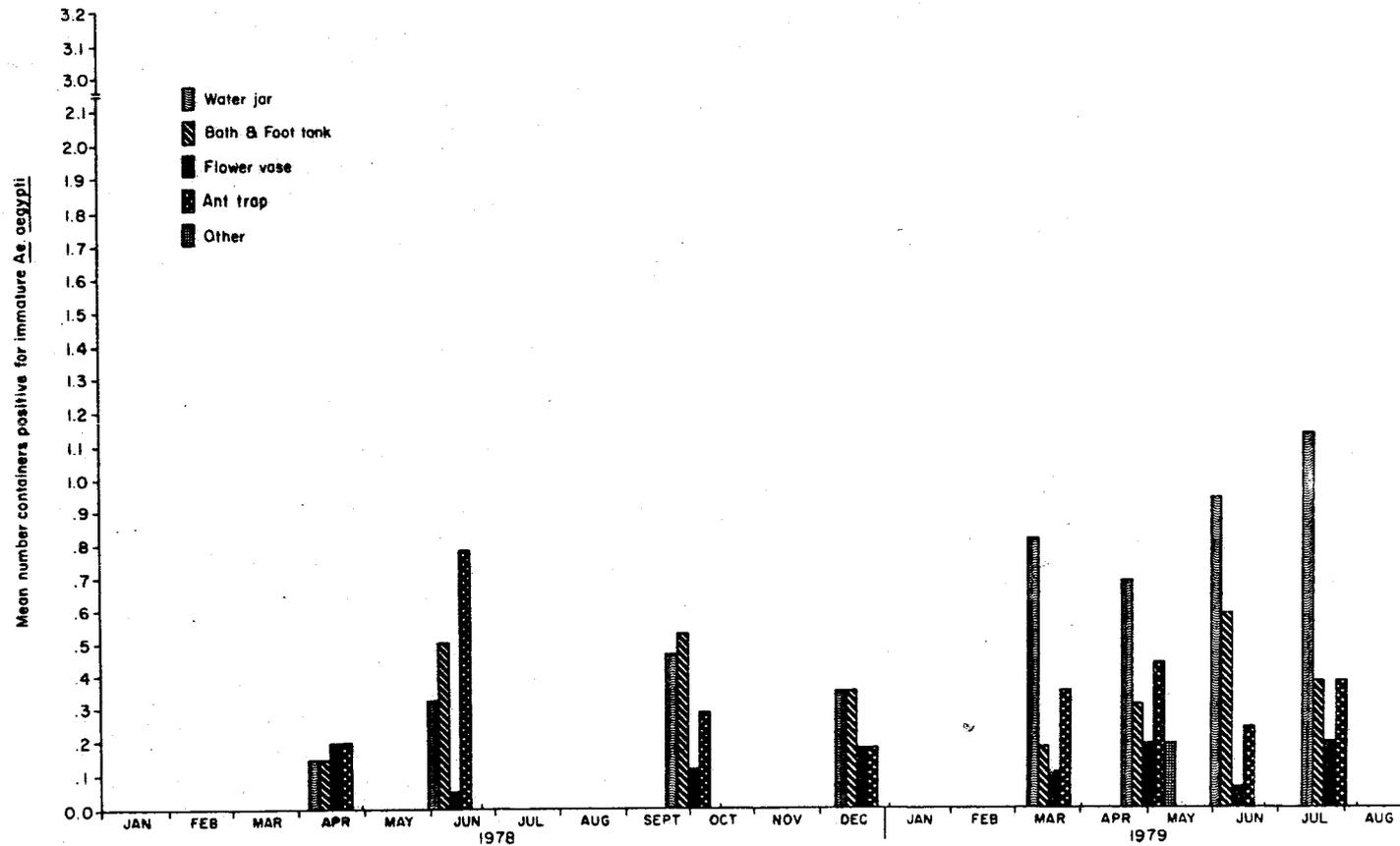


Figure 12. Mean number of containers positive for immature *Aedes aegypti* per shop residence (outside & inside), Din Daeng, Bangkok, Thailand (1978-79)

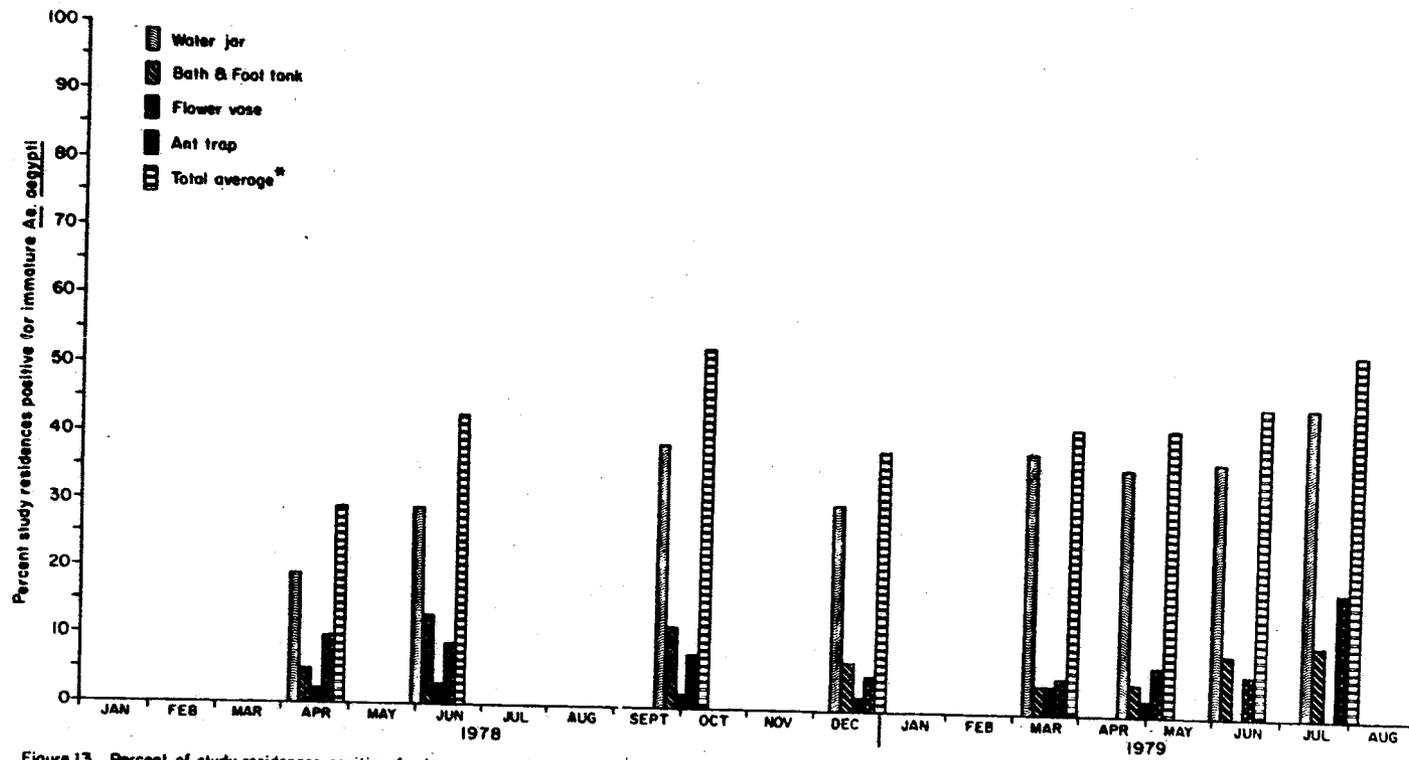


Figure 13. Percent of study residences positive for immature *Aedes aegypti* by different types of containers, Din Daeng, Bangkok, Thailand. (1978-79)

*house positive for more than one type container only counted once.

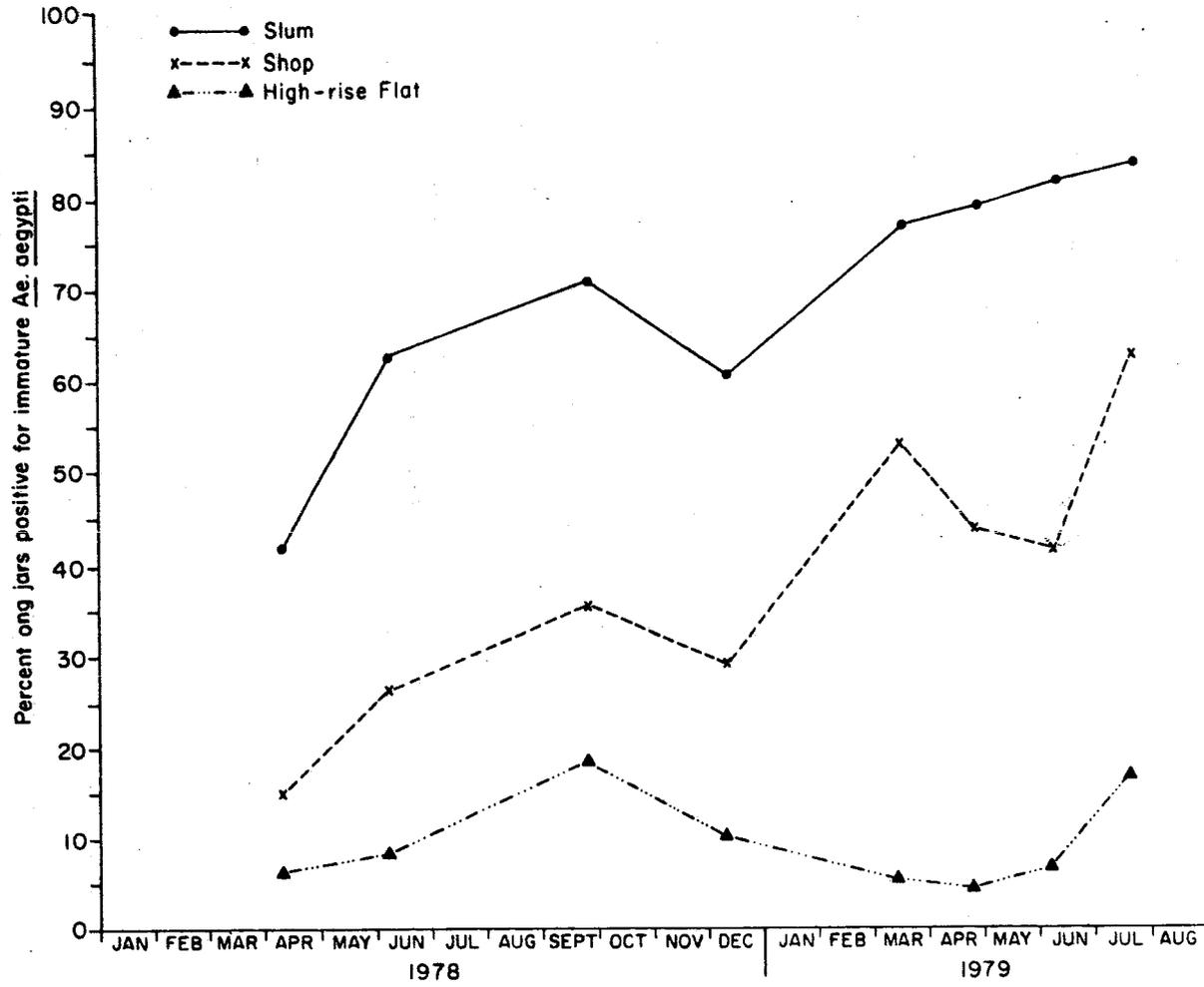


Figure 14. Percent of different type study residences positive inside & outside for immature *Aedes aegypti* for ong (water) jars, Din Daeng, Bangkok, Thailand. (1978-79)

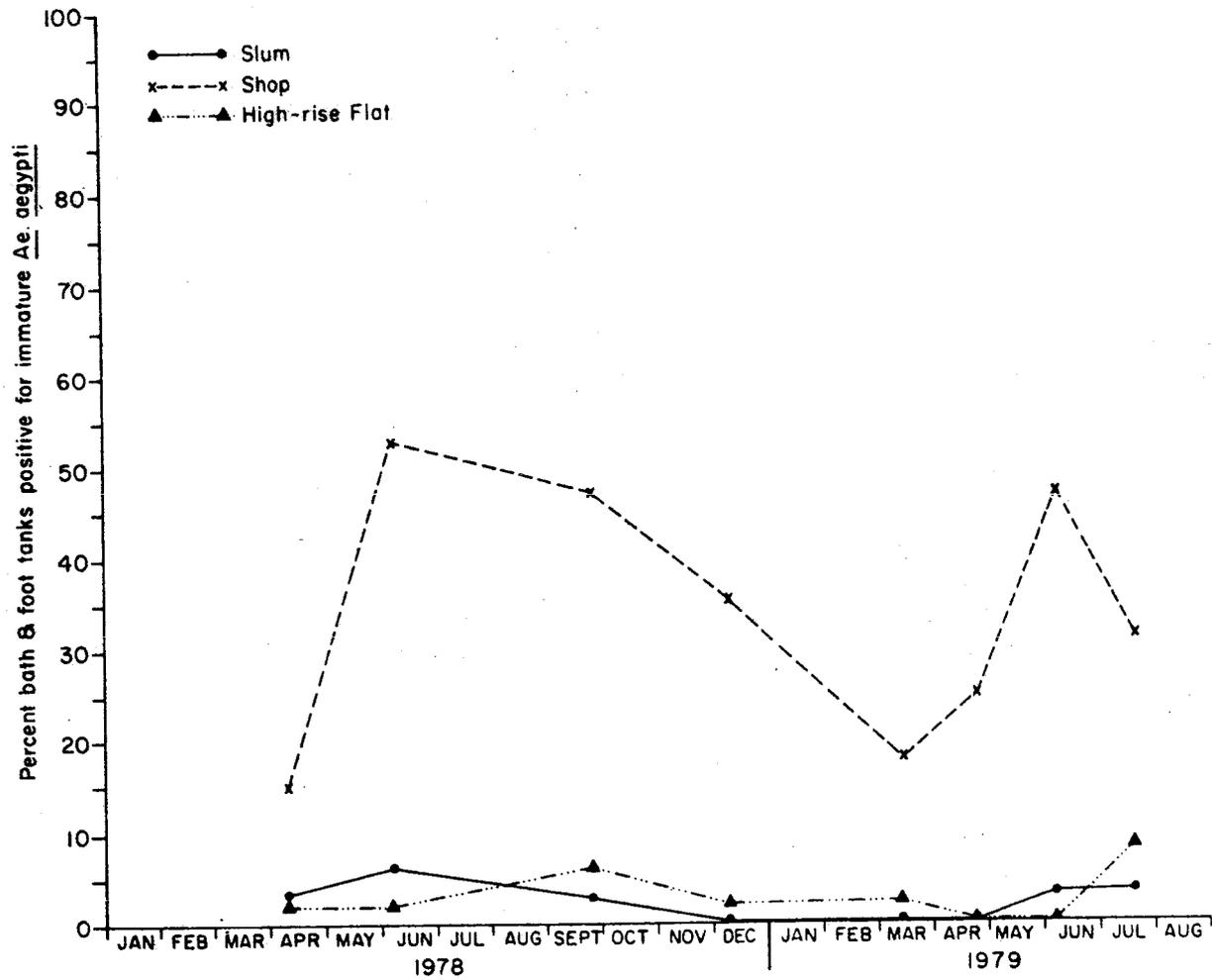


Figure 15. Percent of different type study residences positive inside & outside for immature *Aedes aegypti* for bath and foot tanks, Din Daeng, Bangkok, Thailand. (1978-79)

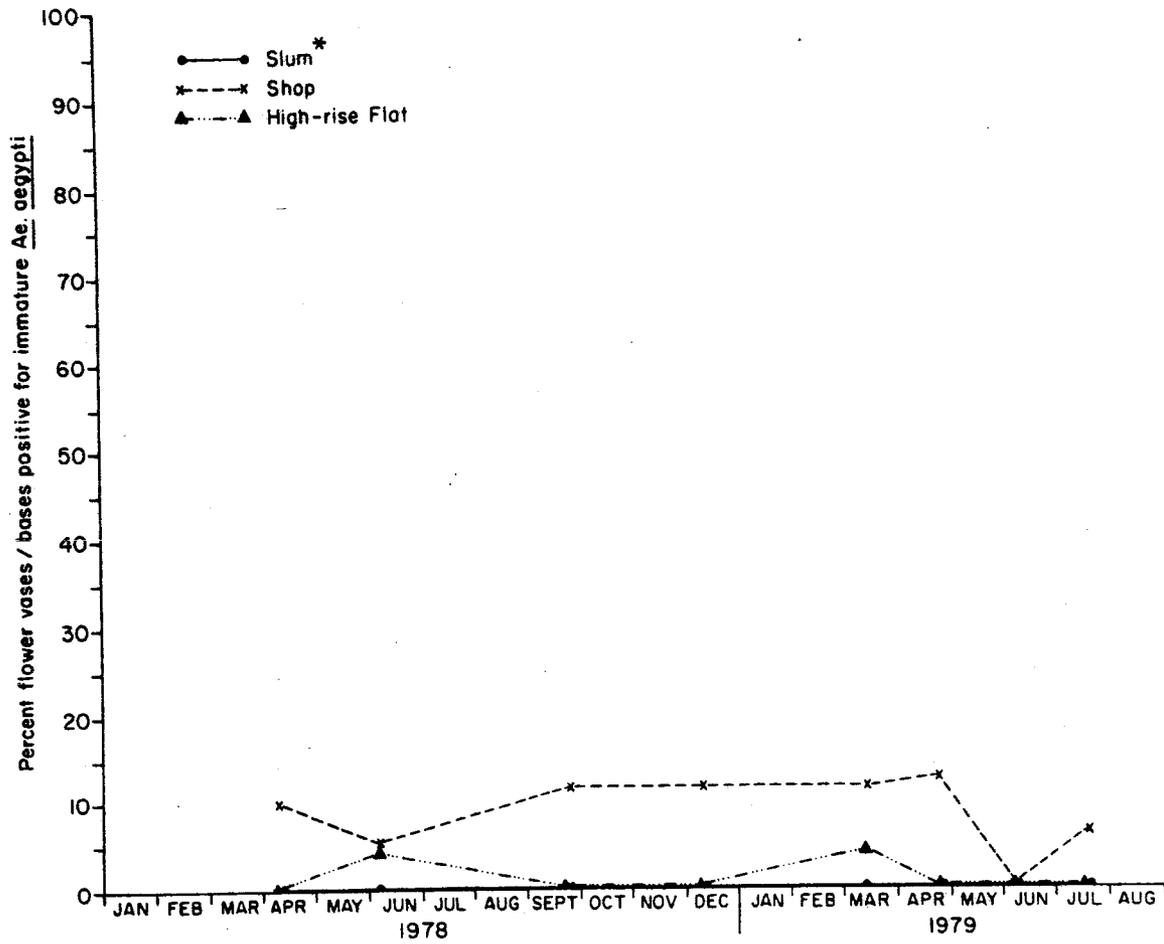


Figure 16. Percent of different type study residences positive inside & outside for immature *Aedes aegypti* for flower vases and flower pot bases, Din Daeng, Bangkok, Thailand. (1978-79)

*All flower vases and pot bases observed were negative.

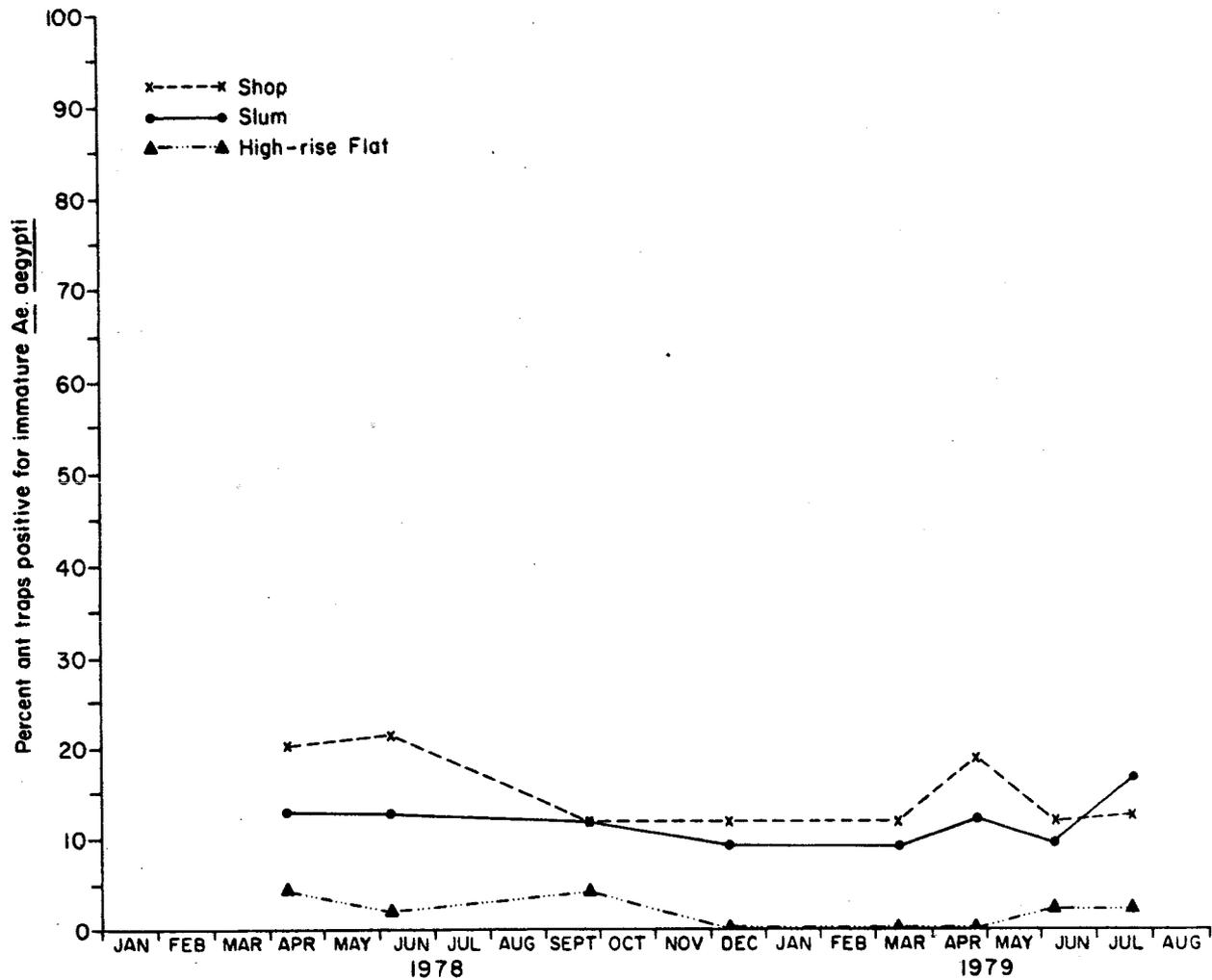


Figure 17. Percent ant traps positive for immature *Aedes aegypti* inside & outside different types of study residences, Din Daeng, Bangkok, Thailand. (1978-79)

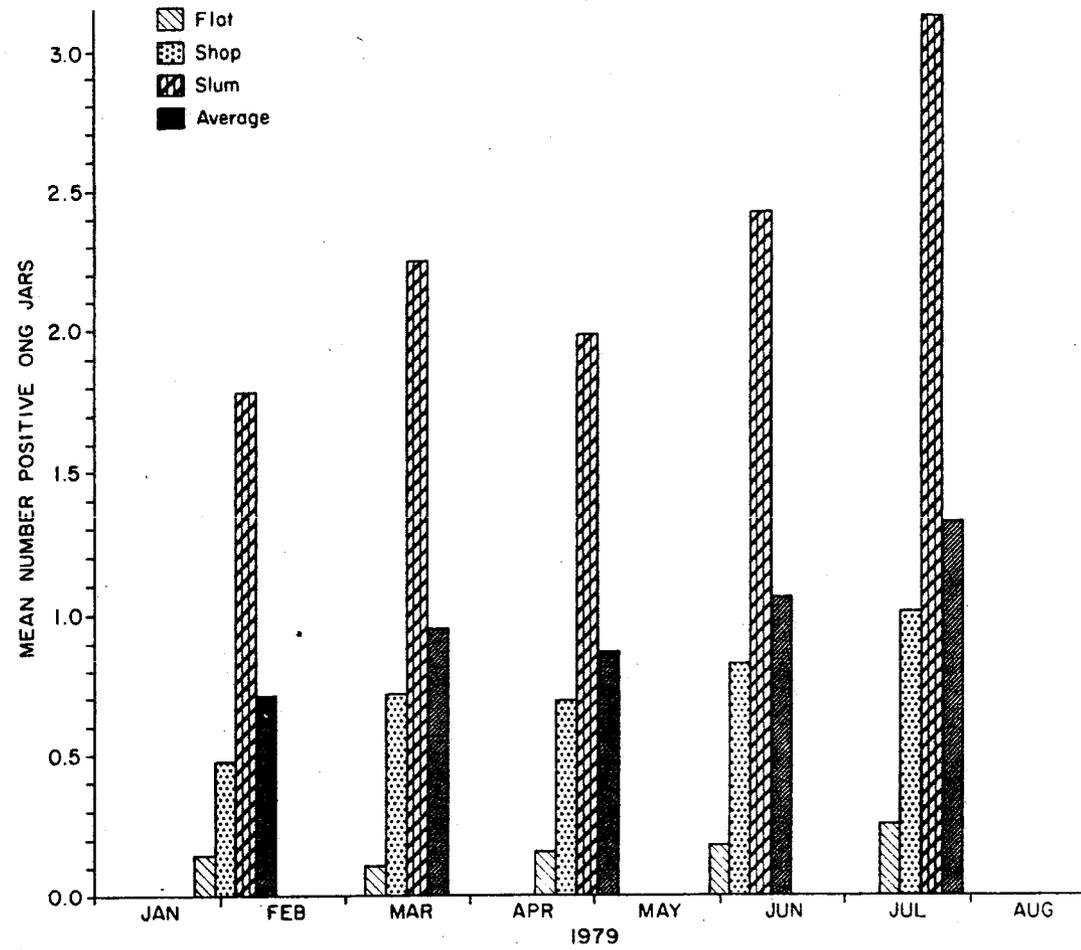


Figure 18. LARVAL TRAP: Mean number of positive ong (water) jars per residence for 3 types of study residences, Din Daeng, Bangkok, Thailand. (1979)

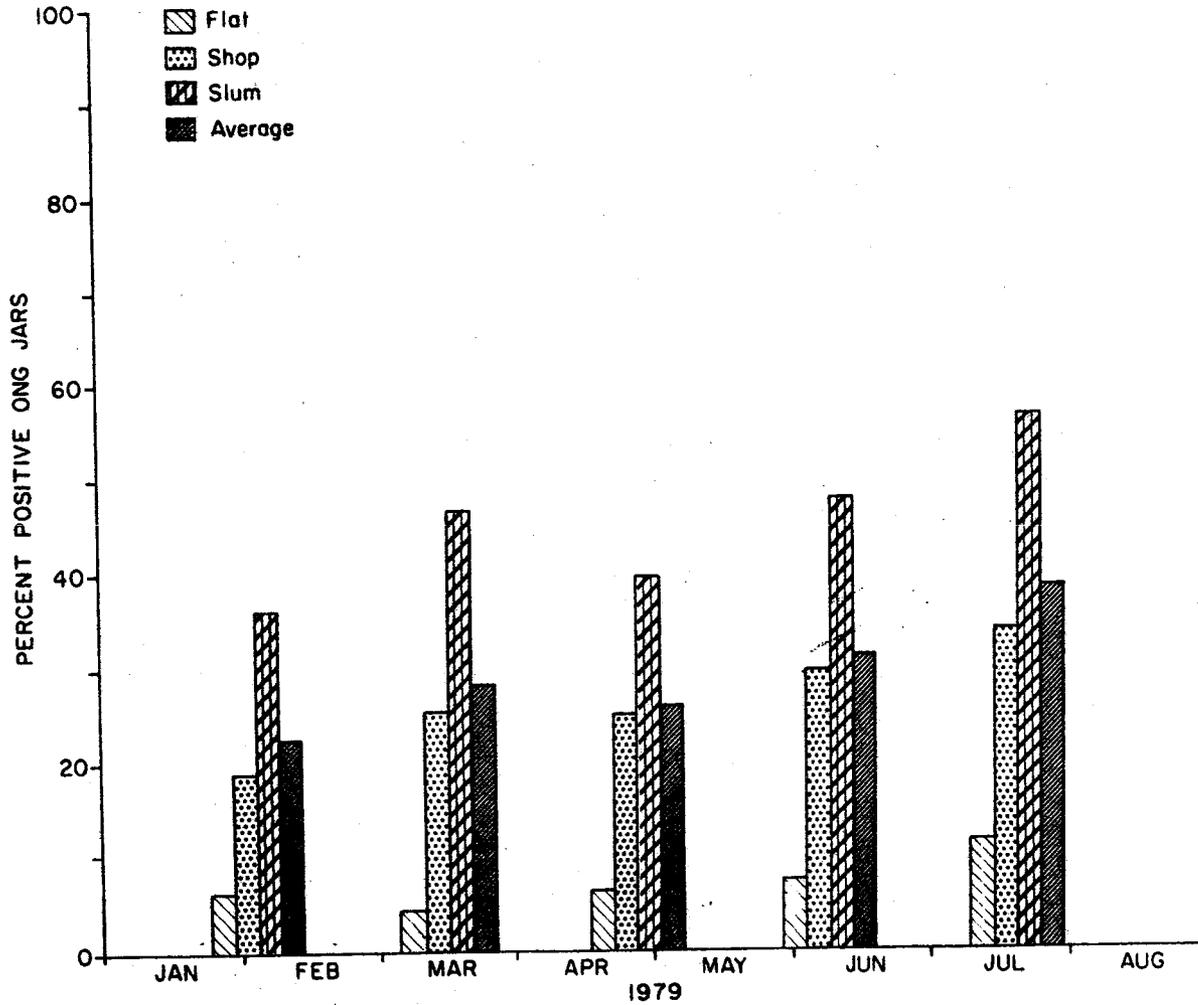


Figure 19. LARVAL TRAP: Percent positive ong (water) jars per total ong jars with water for study residences; Din Daeng, Bangkok, Thailand. (1979)

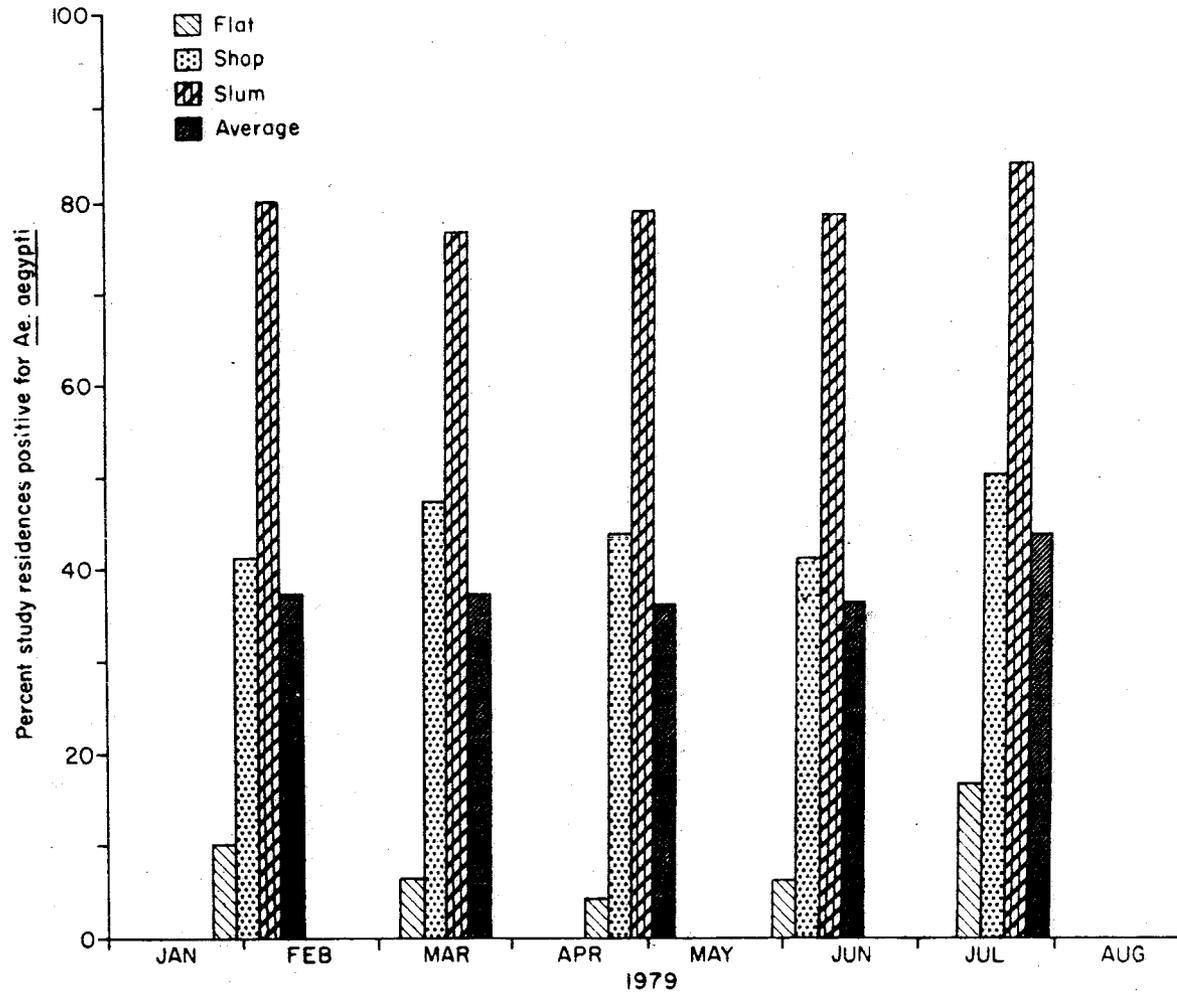


Figure 20. LARVAL TRAP: Percent study residences with positive ong (water) jars, Din Daeng, Bangkok, Thailand. (1979)