Studies on over-wintering of *Culex tritaeniorhynchus* Giles in the Republic of Korea

H. I. REE *
Y. WADA **
P. H. A. JOLIVET ***
H. K. HONG ****
L. S. SELF *****
K. W. LEE ****

RÉSUMÉ.

De 1967 à 1974, la section entomologique de l'Institut National de la Santé à Séoul et de l'Unité de Recherches Ecologiques et de Contrôle des Vecteurs de l'O.M.S. (VECRU) ont cherché en vain les sites d'hibernation potentiels de Culex tritaeniorhynchus *en Corée. Tous les endroits possibles ont été fouillés et, au printemps, la neige carbonique, les tentes en plastique, les pièges lumineux ont été essayés, mais toujours sans succès.*

La première capture faite au printemps est datée du 7 avril, et a eu lieu dans le sud à Pusan, et ce avec une tente et de la neige carbonique. L'hypothèse a été avancée ici d'une recolonisation progressive par le moustique du sud vers le nord des niches écologiques vacantes avec une hibernation probable au moins dans le sud du pays dans des conditions encore ignorées.

ABSTRACT.

In 1967-1968, the entomological section of NIH investigated potential over-wintering sites of C.t. *but were unable to find any adults. Then VECRU continued this work from 1969 to 1974. Caves, cellars, animal holes, culverts, straw piles, stone walls, etc., were searched and in the spring time dry ice, plastic tents and light traps were used, but always without success. The earliest capture of* C.t. *was done on the 7th of April in Pusan with dry ice. Hypothesis is advanced here of south-north recolonization by the mosquito with hibernation at least in the southern part of the country in still unknown conditions.*

In temperate countries, like Korea, Japan and maritime Siberia, the primary vector of Japanese encephalitis is believed to over-winter as the adult and to transmit the virus during the warm part of the year. The reverse is the case in the tropics and to a lesser degree in the subtropics, where *Culex tritaeniorhynchus* is present and active throughout the year.

Knowledge regarding the manner whereby *C.t.* over-winters is essential to an understanding of the fate of JE virus or at least the way *C.t.* repopulates the Korean peninsula early spring and in summer. It is well known that *C.t.* hibernates at the adult stage in Japan and it has been also proved by the WHO Vector Ecology and Control Research Unit (VECRU) that this mosquito does not over-winter in Korea in the larval stage. Searches for over-wintering adult mosquitoes have unfortunately been so far negative in the Korean peninsula.

* *Section Chief, WHO-VECRU, Séoul, Korea.*
** *Professor, Department of Medical Zoology, Nagasaki University School of Medicine, Japan.*
*** *Project Leader, WHO-VECRU, Seoul, Korea.*
**** *WHO-VECRU, Seoul, Korea.*
***** *WHO Entomologist, P.O. BOX 302, Jakarta, Indonesia.*

1. JAPANESE FINDINGS.

Most of the literature published in Japan indicates that *C.t.* usually, but not always, over-winters as a nulliparous (unfed) female and that hibernation is linked with photoperiod and temperature. In nature, *C.t.* has been found in winter in various locations, like brush and wood piles of the common pine, wood piles from other trees, bundled bamboo, caves, etc. (Bullock *et al.*, 1959). Wada *et al.* (1968) found that the *C.t.* collected in caves in the Izu peninsula and Chiba prefecture represented 17.2 % of the *Culex* collected. All, at that time, were nulliparous, a fact which suggests that the virus does not over-winter in the body of the mosquito in temperate climates. The situation is probably different in the northern subtropics, where the insect is partly active even in winter.

Hibernated females were also found in Japan on stone walls, near paddy fields (Omori *et al.*, 1965). In early spring, many over-wintered females can be collected by the dry ice trap in Nagasaki, Fukuoka and Osaka. The adult females kept in a cellar, animal house or other dwelling, over-wintered successfully, but larvae died by the end of the year in an outdoor insectary in Nagasaki.

Japanese workers have found a great longevity for *C.t.* adults (231 days) and have found that females can survive until as late as early June. Fed females can live longer than unfed ones, while males live for shorter periods than the females. Important factors for survival in hibernating sites seem to be absence of lightness and quietness. The start of emergence from hibernation of *C.t.* females occurs earlier in the more southern places of Japan, as in Korea : late March at Nagasaki, early and mid-April in Osaka. As we know from VECRU findings, this happens about mid-April in Pusan and much later in Sintaien and Seoul. From Japanese observations, it may be concluded that the main over-wintering habitat there is in the small underground space in stone walls, and banks of terraced paddy fields.

2. METHODS USED IN KOREA.

A search for potential sites such as rodent burrows, with specially adapted screened traps, upland caves coal or gold mines, subfloor heating spaces, roof tops, thatch, etc., has been regularly done since the first phases of the VECRU project which began in 1969. During each November to February until 1974, culverts and caves near Seoul have been examined for mosquitoes at least twice a month. A sample from each habitat was collected and mosquitoes identified. Often, the mosquitoes collected were dissected and the parous rate determined. Some of the mosquitoes (*Culex pipiens* mainly) were pooled and stored for virus isolation attempts. Usually a team of at least four men worked for several hours on the days searches were made. Over 200 man hours of collection were made each year for over-wintering specimens.

During trips to Southern Korea, and also around Seoul, plastic tents were used above rock piles and the stones dismantled one by one. Heating of the tent was done by using gas stoves in order to induce the mosquitoes to fly. Sometimes, the sun alone was sufficient to raise the temperature on bright, sunny days. Plastic tents were also used over straw piles, reeds and rock walls. It seems necessary to have a temperature of around 10°-12° C inside the plastic tent to induce the mosquitoes to fly about. However, the tent is not fully effective in catching mosquitoes near or on the ground, because the temperature there is still too low. Another short-coming of our technique is the short period of use involving only several hours. With the tarpaulin tent in Japan, this trap was kept on the same area for weeks.

In October and November mosquito larvae were collected from rice fields and swamps around Seoul. Those larvae were placed in containers in the rice field and their development followed until a heavy freeze made further observations impossible. Also attempts were made to collect mosquitoes resting along rice fields and houses in winter.

In early March, further attempts were made by using mosquito nets and dry ice to capture mosquitoes coming out from hibernation during sunny days and at different times of the day. This method was very successful in Japan. In addition, black light traps, cow baiting, etc., were used in different parts of the country in order to detect the first *C.t.* female attracted and, eventually by use of light traps, the first males.

Actually, there are only a few ways to investigate ower-wintering mosquitoes. One, the direct way, is to catch resting mosquitoes during their hibernation (plastic tents, caves, mines, culverts, sweeping nets, etc.); and the second way, indirect, is to induce them to leave their hibernating places early in spring and to attract them with animals or dry ice. The dry ice trap, operated by putting dry ice into warm water inside an open mosquito net, was supposed to be the best technique because mosquitoes attracted in March-April must have been specimens which have hibernated successfully. Another, and third, method, the experimental study of hibernation in vitro, has not been attempted by the VECRU team because of lack of suitable laboratory facilities, but was successfully done in Japan as already mentioned.

When parous rates have been determined for hibernating *Culex pipiens* in Korea, it was found that about 95 % of 3 000 specimens were nulliparous.

3. RESULTS IN KOREA.

Adult activity of *Culex tritaeniorhynchus* in Pusan began generally in April, in Sintaien in May, and in Seoul in July. The adults have always been collected before larvae in all areas. Never, despite intensive searches done before and after the creation of the VECRU unit, was one single specimen of *C.t.* collected in winter in Korea. The earliest capture in spring time was made on 7 April 1973 and that was a *C.t.* attracted by dry ice in Pusan. All those early adults were over-wintered specimens and the first generation larvae were found early in June in Pusan and Sintaien, and in July in Seoul. The species probably appears earlier in Chejudo Island, south of Pusan, but the data obtained are not reliable.

Whang (1961) made a two year survey of the hibernating mosquitoes mainly around Seoul. Seaches were done in numerous types of suitable hibernating places : basements, roofs, barns, air shelters, caves, cement tanks, cisterns, wells, rock cracks, tree holes, bridges, stables, culverts, jars and straw piles. Most of the mosquitoes found were inside caves (15.5 %) and culverts (83,3 %), and *C. pipiens* was the dominant species (97 %). The rest consisted of *Anopheles sinensis, A. sineroides, C. orientalis.* All *C. pipiens* checked for JE virus were negative. A total of 5,262 adults were collected and all were females except for six male *C. pipiens.* No *C.t.* were collected.

In Soosaik, first the malaria team, then VECRU, investigated the over-wintering mosquitoes each year from 1960 to 1974 during the months of November to February. The Soosaik area is situated on the west side of Seoul city in a new developing suburb. The following species of mosquitoes involving about 10,000 females were collected : *C. pipiens,* the most abundant (99 %) and *A. sinensis, C. orientalis* and *A. sineroides,* again no *C.t.* were collected.

VECRU also investigated other areas : deep limestone caves in eastern Korea from January to February 1973, where only *C. pipiens* and *C. orientalis* were captured; coal mines in 1970 and natural rock piles and a mountain pass near Pusan in 1972, all with negative results.

Plastic tents were used when feasible, and rock piles were covered and carefully demolished during December 1972 at Pusan, and only *A. sinensis* (15), *C. pipiens* (31), *C. orientalis* (4), and *A. pullus* (1) were found. Artificial rock piles were also built around Seoul in 1971 without any practical result. The exploration of Wido Island situated 12 km west of the Korean peninsula in the Yellow Sea, once in December 1971 and a second time in March 1972, also led to negative results, despite the fact that there was a heavy *C.t.* breeding in the Island during the summer time in the vicinity of the area tested. Dry ice was also negative in March 1972.

Experimental work was also done with larvae in 1970 and 1971. Some collected from rice fields were placed into large pipes driven into the ground and it was found that emergence of adults continued until the water had a covering of ice, around mid-November; the larvae then died. Searching of houses and animal shelters, as well as sweep net collection in and around rice fields from mid-October to mid-November, did not yield any positive results. Rat holes were also investigated in Pusan in March 1972 with a special mosquito trap. No *C.t.* were captured.

The last survey done by VECRU covered the warm temperate region of the south and the eastern side (Pohang) in March 1974. Some of the areas explored like Pusan, Kohung, Yosu generally showed a very intensive breeding of *C.t.* during summer time. All possible kinds of hibernating places were investigated using various techniques, like plastic tents and dry ice. In an area like Kohung, where the summer breeding in the rice fields and marshes is enormous in August-September, the team explored deep and abandoned gold mines. They were situated along the valleys in what was supposed to be an ideal position. During that trip only *C. pipiens* (87), *A. sinensis* (31), and *C. hayashi* (3) were captured. The mines harboured only *C. pipiens.* Lack of positive results could this time be attributed to the exceptionally cold weather, but nonetheless other species of mosquitoes were captured.

In Korea, as in Japan, *A. sinensis* appear earlier than *C.t.* and *C.t.* appears earlier in the south than in the North. Why, despite an extremely intensive and long search, *C.t.* has not been captured in the winter in Korea is really hard to understand. A partial explanation may be that the species over-winters in extremely small numbers.

4. DISCUSSION.

Despite continuous efforts during five years, the VECRU team was unable to collect any over-wintering specimen of *C.t.* Other species of mosquitoes were collected and often in appreciable numbers. It is, however, assumed that *C.t.* over-winters in the adult stage and that oviposition commences in late spring. A severe or mild winter would influence the number of females that

successfully overwinter, but it is a point as to whether this factor, or a wet mild spring, is the most conductive to the initiation of the summer population build-up. Temperatures must therefore be considered regarding over-wintering and the spring cycle.

It would be easier certainly to find hibernating females in Kyushu Island than in Korea because of the much higher density of the over-wintering population. It would be also interesting to know if *C.t.*, a species known to fly considerable distances, could migrate every year into Korea from southern countries and reintroduce the virus. Also, though improbable, this hypothesis should not be fully rejected while *C.t.* hibernation has not been proved in cold continental Asia. Both factors could be combined : hibernation and migration without contradictory reasons. A problem arises — whether or not the females of *C.t.* keep the JE virus during winter. Not all the females hibernate nulliparous as we have seen, even if the majority do. The chance in keeping the virus that way seems very small, though it was demonstrated that under experimental conditions, *C.t.* females infected with the virus in autumn can over-winter successfully and transmit the virus to susceptible pigs in spring (Mifume 1965). Over 60,000 over-wintered females were examined for the virus in 1965-1973 in the Nagasaki area without success (Hayashi *et al.*, 1966, 1970, 1973). The JE virus isolated in February 1973 from *C.t.* females in Amani-Oshima Island, situated on the fringe of the subtropics, does not mean a stopped feeding activity of the mosquito.

As we know from observations and theoretical calculations (Wada and Orori, 1971) it has been estimated that the number of generations varies in temperate climates from 7.7 in Kagoshima, 7.4 in Nagasaki and 2 in Vladivostock. In Korea, it has been estimated (Mathis and Jolivet, 1974) that the number of generations are about five in Sintaien and probably only four in Seoul, but that number can vary slightly from year to year. One question arises — do the mosquitoes from northern places like Seoul or Manchuria really hibernate on the spot or do they migrate from the subtropical zone or neighbouring warmer countries, such as Japan or China, each spring ? It is extremely difficult to answer that question. South-west Korea is characterized by a mediterranean type of climate, often called subtropical, but is more properly classified as warm temperate because of its evergreen trees (lauretum), the presence of ivy *(Hedera rhombea)* and shrubs like *Camellia*, and a year average of 14 °C.

If there is a southern over-wintering, there is nothing to oppose the hypothesis of a quick northern repopulation and rapid building up of the population. It would not exactly be a migration north, as in the case of the numerous butterflies and moths which every year repopulate Great Britain from breeding done in the mediterranean region, but a spot to spot reconquest of ecological niches left unoccupied by the destruction of the species in November-December. This is purely hypothetical and only a marking of mosquitoes would provide adequate proof, but let us mention that the building up of an enormous population of certain species is much quicker in the arctic zone. Finally, over-wintering of *C.t.* in the northern areas is highly probable, but it has not been proved.

On a practical note, whether it is hibernation or repopulation south-north, there is no point using larvicides at spring time to stop the future rapid population build-up. Experiments done in Japan on a small island have shown that only summer treatment is efficient and spring larviciding does not stop a quick recovery of the mosquito numbers during the transmission season. New research should be done in Korea in the extreme south in areas with heavy breeding, like Kohung, and particularly in the south-western islands, using perhaps new techniques (such as sweeping nets or D Vac) and during warmer weather than experienced during our survey in March 1974.

ACKNOWLEDGEMENTS.

We wish to express our deep appreciation for the efforts of the VECRU national team who helped looking for the hibernated females of *C.t.* in various areas of Korea from November to April. Pioneer work of Dr R. Tonn and Mr Mouchet has also been very useful in directing the efforts of the VECRU team. Mr H. L. Mathis also helped with the last survey.

Manuscrit reçu au S.C.D. de l'O.R.S.T.O.M. le 30 janvier 1976

REFERENCES

BULLOCK (H.R.) *et al.*, 1959. — Notes on the over-wintering of *Culex tritaeniorhynchus* Giles in Japan. *Mosq. News*, 19 (3) : 134-188.

HAYASHI (K.) *et al.*, 1966. — Ecological studies on Japanese encephalitis virus. Isolation of Japanese encephalitis virus from mosquitoes collected in Nagasaki and Kagoshima districts, Japan, in 1965. *Endem. Dis. Bull. Nagasaki*, 8 : 61-73.

HAYASHI (K.) *et al.*, 1970. — Ecological studies on Japanese encephalitis virus. Results of investigations in the Nagasaki area, Japan, in 1968. *Trop. Med.*, II (4) : 212-220.

HAYASHI (K.), *et al.*, 1973. — Ecological studies on Japanese encephalitis virus. Reports of investigations in Nagasaki area, Japan, in 1969, 1970 and 1971. *Trop. Med.*, 15 : 212-224.

MATHIS (H.L.) & JOLIVET (P.H.A.), 1974. — Seasonal and yearly population variations of adult *Culex tritaeniorhynchus* Giles, in Korea. *Document WHO/VBC/74.449,* 7 p.

MIFUNE (J.), 1965. — Transmission of Japanese encephalitis virus to susceptible pigs by mosquitos *Culex tritaeniorhynchus* after experimental hibernation. *End. Dis. Bull. Nagasaki Univ.,* 7 (3) : 178-191.

OMORI (N.), *et al.,* 1965. — Experimental hibernation of *Culex tritaeniorhynchus* in Nagasaki, Japan. *Endem. Dis. Bull. Nagasaki,* 7 : 288-295.

WADA (Y.), *et al.,* 1968. — Mosquitoes over-wintering in Izu Peninsula and Mt. Nokogiri. *Jap. J. Sanit. Zool.,* 19 (1) : 82-83.

WHANG (C.H.), 1961. — Hibernation of Mosquitoes in Korea. *Mosquito News,* 21 (1) : 17-20.