Attack on the Vector of Filariasis in British Guiana

GEORGE J. BURTON, Ph.D.

ALONG the heavily populated coast of British Guiana in northern South America, in a strip about 10 miles wide, about 20 percent of the population of more than 500,000 are infected with filariasis. When a swelling appears, this condition is known as "elephantiasis." "Big leg" disease is very prevalent in British Guiana, particularly in the capital, Georgetown. It is caused by the filarial parasite *Wuchereria bancrofti*, which in many parts of the world is transmitted chiefly by the common domestic mosquito, *Culex* (*Culex*) *pipiens quinquefasciatus* Say, also called *Culex* (*Culex*) *fatigans* Wiedemann and *Culex* (*Culex*) *pipiens fatigans*. This mosquito is also common in the United States. Forty years ago it transmitted filariasis in the southern United States (*1*), especially in the southeast, but gradually the disease died out because of the diminished number of human reservoirs or carriers.

Two other types of filariasis occur in British Guiana, primarily in the native South American Indians, or Amerindians, who live in the interior of the country. One is acanthocheilonemiasis, due to the parasite *Acanthocheilonema perstans* (Manson) Raillet, Henry, and Langeron, and the other is mansonelliasis or Ozzard's filariasis caused by *Mansonella ozzardi* (Manson). The vectors are various species of *Culicoides*. The adult worms are usually considered nonpathogenic, producing few if any pathological changes or symptoms. The study reported here, therefore, did not include these parasites or their vectors, and the term "filariasis" refers only to the elephantoid type which is caused by *W. bancrofti*.

Dr. Burton, a Regular Corps officer of the Public Health Service, was detailed as vector-borne disease specialist (filariasis) to the U.S. Agency for International Development at the time of this study. He was assigned to the filariasis research and control unit in British Guiana. This unit was financed by both the U.S. and British Guiana Governments. He is now chief medical entomologist at the National Institutes of Health–NIHMR Joint Research Program, c/o American Embassy, Accra, Ghana.

Background

In the first half of the 19th century indentured servants were brought to British Guiana from India and Africa to work as laborers on sugarcane plantations. Since filariasis is prevalent in India and in many African countries, the disease was undoubtedly introduced from these sources. In the past half-century, each blood and vector survey made from time to time in British Guiana revealed a considerable amount of filarial infection in both human and mosquito hosts.

Objectives of Study

During the 6 years before the study began in April 1961, many infected coastal residents were treated with diethylcarbamazine. Antimosquito measures had not been attempted since Symes and Hadaway (*2*), Giglioli (*3,4*), and Charles (*5*) had failed to obtain effective control of the vector by indoor residual spraying of houses and latrine walls, using DDT and other insecticides. They all stressed the need for effective antilarval measures.

Not only was it desirable to determine how best to control the vector but also to determine

whether there were any other vectors of *W. bancrofti*, whether the other elephantiasis-causing parasite, *Brugia malayi*, occurred in British Guiana, and whether there were any special factors in the bionomics of the vector which would help in controlling it. In addition, accurate blood and mosquito surveys would add considerably to the existing knowledge concerning the epidemiology of the disease in British Guiana.

The entomological activities which I undertook included collection, identification, dissection, and mounting of adult mosquitoes; collection, identification, and mounting of mosquitoes in aquatic stages from many types of breeding places, and breeding them to adults in an insectary; diagnosis of dissected mosquitoes for filarial parasites; flight range studies; laboratory transmission experiments; transmission hut studies; insecticide resistance tests; and determination and institution of effective control measures against larvae and pupae of *C.p. quinquefasciatus*.

Control Areas

The vector investigations and control measures in this study were carried out primarily in an area about 1¼ by 1½ miles in extent, on the coast about 11 miles due east of Georgetown in Demerara County. This was designated as the "Buxton control area." The heavily infected villages were Buxton, Friendship, and Annandale. Control measures were also carried out in a half-mile barrier zone immediately eastward, including the villages of Vigilance, Bladen Hall, and Strath Spey; and immediately westward in Lusignan. The Atlantic Ocean bordered the northern boundary of the area, and the southern border was mostly uninhabited grassland. All the resources of the filariasis research and control unit were concentrated in this control area in an attempt to control the vector and stop transmission of the disease by combined antimosquito and antiparasitic measures.

No control measures were undertaken in the two other major infected areas in which vector infection studies were made: the Georgetown peripheral villages of Campbellville, Kitty, Lodge, and La Penitence; and the Golden Grove coastal area comprising the villages of Golden Grove, Cove and John, Victoria, Nabaclis, and Plantation Hope.

Breeding Places

In British Guiana, *C.p. quinquefasciatus* breeds primarily in the dirty water of pit latrines or privies and secondarily in the clean water of drums, barrels, simple tanks, and in some other artificial containers. On the other hand, in many other countries this mosquito breeds heavily in cesspools, ditches, drains, catch-pits, coconut-pits, dung-pits, and in other pits which are polluted with human excrement or other organic substance which causes putrefaction (*6,7*). Along the British Guiana coastlands, however, there are outdoor latrines in which the vector breeds heavily, but its larvae or pupae were rarely found in any of the relatively clean trenches, ditches, and drains which run throughout the infected areas.

It became obvious that in British Guiana the major vector prefers to lay its eggs in confined rather than open water. Breeding in pit latrines and septic or other tanks was often so heavy that one dip yielded from 300 to 500 larvae and pupae and numerous egg rafts. Also, before our control measures were undertaken in the Buxton coastal pilot area, 14 to 25 percent of the drums and barrels contained larvae of *C.p. quinquefasciatus*. Generally, no other species was associated with this vector in latrines. In drums, however, *Aedes aegypti* was often present concurrently with *C.p. quinquefasciatus*. Thus control of breeding in drums would eliminate simultaneously the vectors of filariasis and yellow fever.

In prior studies, Giglioli (*4*) had reported that breeding places of *C.p. quinquefasciatus* ranged from drinking water containers to sewers, including pit latrines, cesspools, uncovered septic tanks, sullage, and stable drains. Charles (*5*) indicated that production foci were predominantly water-logged latrines.

Laboratory Development

In white enameled basins containing water from natural sources or latrine water diluted with natural water, eggs of the vector hatched

in 1 to 2 days. Newly hatched larvae developed to the pupal stage in 4 to 6 days, and this stage lasted for 1 to 2 days before the adult emerged. Thus, as has been found by many other investigators, the complete cycle from egg to adult took from 7 to 10 days at 85° to 90° F. and at a humidity of 85 to 90 percent.

When females were completely wrapped in wet towels and kept in mosquito netting cages, they generally lived up to 1 month. Greater longevity, up to 3 months, was obtained when they were kept in lantern chimneys containing a resting grid or screen. Water-embedded paper towels against the bottom netting of the chimneys provided maximum humidity. Ten percent sucrose soaked into absorbent cotton provided nourishment, and aluminum foil covering the cotton helped to retain the humidity. Longevity increased considerably when the chimneys were kept in an air-conditioned room.

Indoor Resting Places

Over a period of 18 months, 21,016 male and female (mostly female) *C.p. quinquefasciatus* were collected during mornings and evenings from houses in 28 villages. Of this number 8,384, or about 40 percent, were found resting on walls; 6,091, or 29.0 percent, rested above the 3-foot level, while 2,293, or 10.9 percent rested below the 3-foot level. There were 7,340, or about 35 percent, found on hanging objects, and 4,928, or 23.4 percent, were collected behind or under furniture including bookcases. For comparison, Chow and Thevasagayam in Ceylon (*6*) found 6 percent resting below the 1-meter level on walls and 17 percent above 1 meter, or a total of 23 percent on walls; also 60 percent on clothing and other hanging objects, 9 percent on furniture, and 8 percent beneath the roof. Deane (*8*), working in Brazil, found 70 percent on walls, of which 20 percent rested on the upper portions and 50 percent on the lower portions; the remaining 30 percent rested behind and under furniture and on clothing.

In Buxton before the control measures were instituted, collectors were stationed at windows at dusk to catch the adults as they flew through the window. These vectors began entering houses about 7 p.m., and fairly good numbers entered between 9 p.m. and 10 p.m. They fed readily on entering, even in lighted rooms and on moving arms and legs. After the blood meal they generally flew to the wall, but some flew out the window. Giglioli (*3*) likewise had found that houses were a favorite resting place of these vectors, many of which were males. He also found that 66 percent of the females caught in houses were engorged, and that they attacked man and animals indiscriminately both indoors and outdoors (*4*). In Lusignan on the east coast, close to Buxton, Giglioli had found that the highest influx into houses occurred between 4 a.m. and dawn.

Vector Infection Rates

During this study, 15,622 *C.p. quinquefasciatus* obtained from 679 houses were dissected. Of this number, 1,514 had *W. bancrofti* parasites, an overall infection rate of 9.6 percent. Only 80 or 0.5 percent, of the naturally caught 15,622 vectors had infective larvae in any part of the body. Generally only one or two infective larvae were found in contrast to five infective larvae usually found after laboratory feeding experiments.

From quarter to quarter in the coastal Buxton control area, vector infection rates dropped considerably after latrine spraying operations and mass chemotherapy with diethylcarbamazine. In Buxton and Friendship the initial mosquito infection rate was as high as 54 percent. As a result of the combined control measures this rate dropped to 4.3 percent.

Other overall infection rates during the period were: Kitty, 10.2 percent; Campbellville, 11.4 percent; Lodge, 10.4 percent; and La Penitence, 9.7 percent. Any person residing in Georgetown proper could easily be infected from this constant reservoir of infected mosquitoes on the edge of the town, within easy flight range of the vector. In the Golden Grove area the overall vector infection rates were: Golden Grove, 13.5 percent; Cove and John, 13.0 percent; Victoria, 11.1 percent; Nabaclis, 8.4 percent; and Plantation Hope, 11.7 percent. The general vector infectivity rates were about the same as the overall total, 0.3 percent to 0.8 percent, with an average of 0.5 percent.

No *Brugia malayi* was seen during this entire study.

Other Vectors

Of a total of 41 additional mosquito species found in the filarial areas, two other species were found with advanced stages of *W. bancrofti; Mansonia* (*Mansonia*) *titillans* Walker and *Anopheles* (*Nyssorhynchus*) *aquasalis* Curry. I had also found the latter species to be the vector of coastal malaria in British Guiana in 1961. Development of the parasite in these two species was found to be approximately the same as in *C.p. quinquefasciatus.*

M. titillans was found to be seasonal, appearing in sporadic numbers in the Buxton control area. It preferred to feed during the day, luckily when there were no microfilariae in the peripheral blood of infected persons. When it fed at night on an infected person with positive blood, the parasite developed readily in its thorax. Because of its diurnal feeding habits, therefore, this species does not appear important in transmitting filariasis in British Guiana. Also, its seasonal habits reduce the numbers which may become infected. On the other hand, *A. aquasalis* was present in large numbers in the filarial areas, and fed at night. Time did not permit the study of its importance as a vector of filariasis, but since the parasite also develops readily to the infective stage in this species, a followup study is indicated. Antimalarial spraying would in a sense also be antifilarial in this dual vector.

Special Studies

Flight range. Various lots of between 1,000 and 1,500 laboratory hatched females were coated with fine gold or aluminum dust prior to being released in the Campbellville and Buxton areas. First, the inside of a 6-inch by 1-inch test tube was coated with the metallic dust by placing a quantity of the dust on a spatula and aspirating it into the opening with a large ear syringe. Forty mosquitoes at a time were released into the coated tube, and the procedure repeated. The agitated mosquitoes were thus well coated with the dust. They were transferred to holding cages until their release the same evening in a location where many persons had *W. bancrofti* microfilariae in their blood. House collections were made in the morning and evening every day for the next two months, in ever increasing circles from the point of release. The metallic reflection made identification of the recovered mosquitoes easy. Data ascertained from this procedure were (*a*) flight range and dispersion, (*b*) the rapidity with which sterile mosquitoes pick up microfilariae from infected persons, and (*c*) development time of the parasite in the vector under natural conditions, providing the infected blood meal was taken on the first night of release. Tests were carried out also to ascertain whether metallic dust could be transferred from a dusted to a nondusted mosquito. Numerous undusted males were kept in the same cage with dusted females, but no case of such transference was seen.

Following four field release trials, it was found that the flight range or dispersal of *C.p. quinquefasciatus* males and females was as much as 900 yards, or approximately one-half mile. Recovery at this distance was made 3 weeks after release. It is probable that with a strong wind dispersal would be increased. The best recovery was made when the mosquitoes were released on a clear, still night in the dry season. Recovery was generally poor when rains followed their release. Depending on the availability of microfilarial blood, there was considerable variation in the infected condition of the recovered mosquitoes, but as might be expected development of the parasite in the vector in nature was slightly more accelerated than in a simple insectary. Sausage forms were found in some recovered mosquitoes 50 hours after their release, which is somewhat earlier than in laboratory-fed mosquitoes.

Parasite development in the vector. Laboratory transmission studies were undertaken to follow the development of the parasite within *C.p. quinquefasciatus*, *M. titillans*, and *A. aquasalis.* In *C.p. quinquefasciatus*, depending on humidity and temperature of the insectary, development of the parasite to the infective stage took from 12 to 16 days or even longer following ingestion of the microfilariae. Higher temperatures led to longer development times. Comparing rapidity of development, the earliest first-stage sausage forms were found in *C.p. quinquefasciatus* between 54 and 57 hours after feeding on infected blood, and in *M. titillans* at 5½ days. In *A. aquasalis* the parasite developed to the

infective stage with the same rapidity as in *C.p. quinquefasciatus.*

Transmission hut studies. A portable, disease-transmission study hut was constructed of plywood and slotted angle-iron or "Dexion" in order to study the uptake of microfilariae by *C.p. quinquefasciatus* under natural conditions. Volunteers having high microfilarial blood counts slept in the hut at night, and vectors were collected in the morning. Ninety female *C.p. quinquefasciatus* entered the hut over 18 nights. Louvered one-way windows prevented their exiting. Of the 90 mosquitoes, 76 (84.4 percent) had fed during the night and 56 (73.7 percent) of these became infected. The microfilarial counts in the fed mosquitoes varied considerably, from 1 to 257, depending on the size of the blood meal from a donor who had an average of 100 microfilariae per 20 cubic millimeters of blood. Some mosquitoes which had fed only slightly or partially did not pick up any parasites. Also, there was evidence that fully fed mosquitoes which had had a blood meal from an uninfected person flew into the transmission hut and were collected along with the others in the morning. This may have been true for about 8 of every 100 mosquitoes which entered the hut while the infected donor was sleeping, therefore a slight correction factor is necessary in interpreting the results. On individual nights 33.3 to 100 percent of the mosquitoes collected from the occupied hut had microfilariae in the stomach or thorax, or both.

Simultaneously, a detailed study was made of various factors in 37 houses near the transmission hut from which 329 infected mosquitoes had been collected during nine mornings. Blood specimens, taken from all the occupants of these houses, were smear-tested. The human infection rates were compared with the mosquito infection rates. Matching infected mosquitoes against infected persons, house by house, it was found that only 13 (35.1 percent) of the 37 houses had persons with positive smears as well as infected mosquitoes. In 24 houses (64.9 percent) which had positive mosquitoes, none of the occupants had positive smears, indicating that the vectors had become infected elsewhere. Only 2 (0.6 percent) of 329 infected mosquitoes had infective larvae, which checks fairly well with the overall infectivity rate of 0.5 percent. In 21 of the 37 houses the percentage of infected mosquitoes varied from 3 percent to 25 percent; whereas in 10 houses 28.6 percent to 50.0 percent of the mosquitoes collected were infected.

Insecticide resistance studies. Tests were carried out with the World Health Organization larval resistance testing kit against larvae and pupae of *C.p. quinquefasciatus.* In each test 25 late third and early fourth instar larvae were used, which had been collected from pit latrines in Campbellville and Lodge in the Georgetown periphery. In separate tests 25 pupae alone were used. Standard dilutions were made using the DDT, dieldrin, BHC, malathion, diazinon, and Baytex provided in the kit. For comparison, tests were also made with plain kerosene, plain gas oil, and plain heavy diesel oil. The most effective insecticide against both larvae and pupae was Baytex, but it was also found that plain gas oil killed larvae and pupae much more rapidly, had excellent surface-holding qualities and a very durable film, and was quite inexpensive and readily available in British Guiana. Also, there need be no concern about development of resistance to the oil. In India Ramakrishnan and associates (*9*) had likewise preferred oils to synthetic insecticides for antilarval operations against this vector.

Resistance of female *C.p. quinquefasciatus* was also tested, using the WHO adult resistance testing kit. In each test 15 mosquitoes were exposed to dieldrin or paper impregnated with DDT for 1 hour, then kept in a holding cage for 24 hours. The same results were obtained with both wild-caught and laboratory-hatched mosquitoes. It was found that 40 to 50 percent of the mosquitoes were resistant to 4 percent DDT and to 1.25 percent and 2.50 percent dieldrin, thus verifying previous reports that adult *C.p. quinquefasciatus* were resistant to DDT and dieldrin. Several tests with 4 percent dieldrin and 5 percent DDT gave the same range of results.

Control

All previous attempts by other workers to control *C.p. quinquefasciatus* by indoor residual spraying had failed because of resistance to the insecticides used. Giglioli (*4*) used 5 percent DDT in kerosene and 10 percent DDT suspen-

sion. Houses were sprayed every 6 to 10 months and latrine superstructures and contents every 3 to 4 months. Unaffected adults were caught even immediately after spraying, and a very definite increase in numbers as well as vigorous larval breeding was apparent 2 months after spraying.

Symes and Hadaway (*2*) controlled the adults with a Hochberg-LaMer aerosol or fog generator using approximately 7 percent DDT. They found considerable resistance of this mosquito to indoor residual 6 percent DDT emulsion and 5 percent DDT solution in kerosene applied at the rate of 2 or 4 quarts per 1,000 square feet. Charles (*5*) attempted control with indoor residual DDT-kerosene solution with and without 2 percent chlordane, and with Gammexane (6.5 percent gamma) BHC wettable powder, Gammexane-kerosene, and chlordane-kerosene. He found appreciable reduction of adults for up to 10 weeks with all formulations. Chlordane as a 3 percent solution in kerosene and 2 percent solution in DDT-kerosene was far more effective than either DDT-kerosene or DDT suspension. The BHC formulations showed no advantage over the DDT preparations. There was no reduction in the number of pit latrine production foci. Charles agreed with other workers that eradication attempts against *C.p. quinquefasciatus* would have to include antilarval measures.

After having determined that plain gas oil was quite effective against aquatic stages of this vector, I found, after field trials, that by spraying 18 swathes (about 23 ounces) of gas oil at one time on the surface of the latrine contents, a durable film resulted which was effective in controlling larvae and pupae for 4 to 6 weeks, and even up to 8 weeks in some instances. A once-monthly latrine spraying program was therefore set up in the pilot area which had 2,027 latrines. The cost of the 23 ounces of gas oil was 5.8 British West Indian cents, equal to 3.5 U.S. cents. The monthly cost of the oil for the 2,027 latrines was therefore $117.57 in B.W.I. currency, or $70.94 in U.S. dollars.

Simultaneously with spraying, each person in the control area received a full course of diethylcarbamazine to kill the microfilariae. Thus antimosquito measures were accompanied by antiparasitic measures. Control of adults was not considered because of their known resistance to the commonly used insecticides. Contrary to conditions in many countries, there was no problem here concerning transportation of the oil, since the Buxton control area was only 11 miles from the Georgetown headquarters. Every working day each spraying team carried only enough oil in jerry cans for 1 day's latrine spraying. This was found to be a simpler procedure than carrying along a full drum of oil each day.

After 1 year of monthly residual larviciding, the effectiveness of this measure was demonstrated by (*a*) total elimination of breeding of the vector in the pit latrines in the control area, as determined by repeated dipping, (*b*) reduction in breeding in drums and barrels adjacent to the latrines, (*c*) great reduction in the number of mosquitoes available for picking up microfilariae, and (*d*) considerable reduction in the average number of mosquitoes per house, so that it was often difficult to find them in many houses.

Before latrine spraying was undertaken, the vector bred in 14 to 25 percent of the drums in Buxton and Friendship. After 1 year this figure dropped to an average of 4.7 percent, based only on drums containing water. There were from 1 to 6 drums in each yard. A team was assigned to empty and turn upside down all unused drums and barrels. Spraying of drums could not be considered because the water was used for drinking and cooking. The high cost of providing mosquito-proof drum and barrel covers prohibited this means of control, but in any event the associative reduction in breeding through latrine spraying counteracted the tendency to increased breeding in drums.

An environmental sanitation program which would include provision of piped water and replacement of pit latrines with cement slab latrines would serve to eliminate breeding foci permanently under the conditions described in the Buxton control area. It is possible, however, that in such case the vector might begin breeding in the many trenches and drains which at present are completely free of such breeding in British Guiana. With the use of tube wells or outside faucets, however, care would have to be taken that no waste water pools remain on the ground, as these have served in some coun-

tries as main breeding places for *C.p. quinquefasciatus* (*10*).

Summary

In British Guiana, during a study which began in April 1961, *Wuchereria bancrofti* was the only filarial parasite found to be causing elephantiasis, and *Culex pipiens quinquefasciatus* was determined to be the primary vector. The parasite developed readily in *Mansonia titillans* and *Anopheles aquasalis*, which were found infected in nature, and they were considered secondary vectors. No *Brugia malayi* was found.

In the coastal Buxton control area, 11 miles east of Georgetown, *C.p. quinquefasciatus* bred primarily in the dirty water of pit latrines and secondarily in the clean, confined water of drums and barrels near latrines. No breeding occurred in the relatively clean, open water of trenches, ditches, and drains.

In an 18-month period, about 21,000 *C.p. quinquefasciatus*, mostly females, were collected from houses in 28 villages. About 40 percent were found resting on walls, 35 percent on clothing and other hanging objects, and 23.4 percent on or under furniture.

On dissection of the naturally caught vectors, about 9 percent were found to have *Wuchereria bancrofti* parasites. About 0.5 percent had infective larvae in their bodies, but generally only one or two of these larvae were found per mosquito.

C.p. quinquefasciatus, hatched in the laboratory, were coated with metallic dust and released. Their flight range was determined to be one-half mile.

Resistance of the adult vector to DDT and dieldrin was confirmed. Effective control was achieved by spraying 23 ounces of gas oil, as a residual larvicide, once a month on the surface of each latrine's contents. Complete elimination of breeding in pit latrines resulted in an associative reduction of breeding in barrels and drums.

Before larviciding was undertaken, the vector infection rate in some parts of the control area was as high as 54 percent. After 1 year of larviciding, this rate dropped to 4.7 percent.

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