WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

File WHO/EBL/66.69
WHO/FIL/66.63
WHO/Vector Control/66.204

ORIGINAL: ENGLISH

INTEGRATED CONTROL AND AEDES POLYNESIENSIS: AN OUTLINE OF THE TOKELAU ISLANDS PROJECT, AND ITS RESULTS

by

Marshall Laird
Chief, Environmental Biology
World Health Organization, Geneva

Nukunono, the largest of the three atolls comprising the New Zealand Tokelau Islands Dependency, is situated some 500 km N of Western Samoa. Its reef, 38 km in circumference, bears many islets of varying size but totalling only 546 hectares in area. The sister atolls, Atafu and Fakaofo, respectively lie 91 km NW and 64 km ESE of Nukunono. Wuchereria bancrofti is present, the vector being Aedes polynesiensis. With the exception of Aedes vexans nocturnus (present only on Fakaofo) this is the only mosquito found in the Tokelaus.

Earlier ecological studies at Nukunono had indicated that the local population of this container-breeding mosquito was free from harmful parasites or significant arthropod predators. In particular, pathogenic fungi of the genus Coelomomyces had not been found there. These organisms, which are virtually restricted to mosquito hosts, are of importance in some other areas among the factors regulating Aedes (Stegomyia) populations in nature. Infected larvae usually die from a combination of causes including damage to internal organs, physical effects of the development of quantities of fungal sporangia, and starvation due to competition for nutrients with their parasites (Madelin, 1965).

Their small size, extreme isolation, limited fauna and flora, and the restricted range of freshwater habitats[1] they afford to a filariasis vector singularly free from natural enemies, all favoured the choice of the Tokelau Islands as the site of WHO's first pilot project[2] directed towards the eventual development of integrated methodologies for mosquito control.

This project commenced in September 1958, with the introduction of a Singapore strain of Coelomomyces stegomyiae (derived from Aedes albopictus) into Nukunono. The fungal inoculum consisted of (1), bottom debris from laboratory containers in which the sporangia-rich remains of parasitized A. albopictus larvae brought in from the field, had been allowed to accumulate; (2), sediment from a Singapore tree hole from which infected larvae had been taken for several months (all the material concerned in these first batches having first been treated to induce the hatching of any insect eggs present, to preclude the possibility of any inadvertent introductions taking place); (3), the bodies of parasitized A. albopictus larvae individually dried on to pieces of filter paper; and (4), living A. albopictus larvae exhibiting C. stegomyiae sporangia (these larvae either died in transit or were killed on arrival at Nukunono, their bodies yielding a rich concentration of what was hoped would prove infective material).[3]

Over a three-week period, every islet of the atoll was systematically searched for mosquito larval habitats. None of the A. polynesiensis larvae sampled throughout the operation proved to harbour any parasites other than the cosmopolitan protozoan Lankesteria culicis (generally regarded as non-pathogenic, and of high incidence in Stegomyia populations wherever it is searched for). In all, 761 larval habitats of a permanent or semi-permanent nature (notably tree holes, including many of the man-made reservoirs hollowed into the lower part of the bole of coconut palms, and locally termed "tungu") were located and seeded with the fungal inoculum. It was

[1] The bulk of these are natural and artificial containers of various types. Surface water is lacking except in crab burrows, the taro marsh in which A. v. nocturnus is established on Fakaofo, and a few very brackish seepages unsuitable for either this mosquito or A. polynesiensis.

[2] Undertaken with the co-operation of the New Zealand Government.

[3] The inoculum did in fact produce infections in second and third instar A. polynesiensis larvae kept under observation at the field laboratory on Nukunono. Although these larvae all died prematurely, evidence was thus obtained of the susceptibility of this host to Coelomomyces, the only such evidence procured during the three weeks spent at the atoll in 1958.

estimated that this represented at least 80 per cent. of such habitats existing at the time of the visit. Periodic microscopic examination of the different batches of inoculum proved that apparently viable Coelomomyces sporangia remained plentiful until the end.

Baseline A. polynesiensis population estimates (adult females, and aquatic stages) were also undertaken at Nukunono, as was a microfilaria survey. For the latter, as well as the usual Giemsa-stained thick films being made from each of those concerned, immediate examinations of fresh blood were undertaken by means of a McArthur field microscope. Mosquito field population estimates and microfilaria surveys were duly repeated on Fakaofo and Atafu, the former atoll being used solely for experimental control purposes. In due course, full data on these activities will be presented in the definitive account of the pilot project,[1] but for present purposes it suffices to record the over-all results of the adult female A. polynesiensis count:-

Atoll	No. of catches (each of 15 minutes duration and involving an unprotected volunteer and a repellent-protected collector)	No. of mosquitos attempting to bite	
		Range	Mean
Nukunono	19	3-255	61.8
Fakaofo	14	10-187	65.4
Atafu	19	7-524	123.1

After a five-day stay at Fakaofo, three weeks were spent at Atafu, where the Nukunono biological control experiment was paralleled by one involving a larvicidal procedure. This called for the use of dieldrin-cement briquettes, each weighing about 20 g and made up from the formula: cement, two parts, coral sand five parts, dieldrin (50 per cent. w.p. powder) three parts. Once again all islets of the atoll were visited, but as it was desired to obtain as high a kill as possible, a briquette was placed in each larval habitat discovered without regard to its permanence. In all, 6500 such habitats (of which more than half were rat-gnawed coconuts, especially prevalent at Atafu and a major source of A. polynesiensis production there) were

[1] To be published jointly with D.H. Colless and other collaborators (the 1958 data herein are extracted from an internal WHO document, by Laird and Colless, comprising the first report on this project).

treated, among them being 125 drums and tanks. Most of the latter were used for domestic water storage purposes in the village itself. A larval mortality of 100 per cent. was invariably noted in any mosquito habitat the day after a briquette was introduced.

Towards the end of 1959, the New Zealand authorities arranged for an interim report on the progress of the above experiments. Based on a very brief visit to the Tokelaus, this provided the first evidence that Coelomomyces has indeed become established at Nukunono (two of 11 "tungu" containing A. polynesiensis yielded 27 parasitized larvae), and revealed the continuing effectiveness of the dieldrin-cement briquettes at Atafu. On this atoll 31 domestic water drums and tanks still containing briquettes were without any mosquito larvae, while 25 of 49 such receptacles lacking briquettes harboured developing A. polynesiensis. A number of briquettes were taken away and forwarded to Singapore, where Dr D. H. Colless (who, together with the author, had initiated these experiments in the Tokelaus in the previous year) tested them against A. albopictus larvae and confirmed that they were still 100 per cent. effective larvicidally despite the fact that they had spent 13 months submerged in water.

WHO supported a more extensive assessment survey in the Group in April 1960. At Nukunono, 118 of 667 "tungu" and natural tree-holes seeded with Coelomomyces inoculum in 1958, were re-located. Sporangia-packed larvae were collected from eleven of these, viable resting sporangia being identified in the bottom debris of three more. Furthermore, proof of natural dispersal from hand-seeded habitats was obtained, for parasitized larvae were found in two halved coconut shells (which from their condition obviously post-dated the first visit) a short distance from three Coelomomyces-positive "tungu". It was thus evident that the fungal pathogen was now established at a level well above that prevailing in nature in Singapore, where the preliminary field work in 1958 had revealed parasitized larvae in just 48 (2 per cent.) of 2454 containers; for considering only the 118 re-located seeded habitats mentioned earlier, and leaving aside those exhibiting viable sporangia but no parasitized larvae, 9.3 per cent. were positive.

All the 1960 Coelomomyces findings concerned only three of the islets of Nukunono atoll. On these, biting collections yielded appreciably fewer adult A. polynesiensis than had been the case 18 months previously. Considering the atoll as a whole, the 19 adult catches made in 1958 (see table, page 3) were repeated at the same sites and same times of day, the 1960 mean being only half that of the former one.

At Atafu, 23 of the village water drums still held dieldrin-cement briquettes. Twenty-two (96 per cent.) of these were free from A. polynesiensis, while of the 103 other domestic containers located, only 18 (17 per cent.) lacked larvae. It is of interest to note that combining the 1960 figures for briquette-free 44-gallon drums at Atafu with those for untreated drums at Fakaofo and Nukunono, 122 (85 per cent.) of the total of 143 held A. polynesiensis larvae. It would seem, therefore, that the 1959 party (having recorded larvae from only 51 per cent. of the briquette-free household water containers) may have overlooked some light infestations; many of the drums and tanks searched for larvae in 1960 held very few larvae, which were located only after a prolonged search using a powerful torch.

In many cases, household water containers in the Tokelaus are replenished two or three times a week from the large central village tank (for which the church roof acts as a catchment area). Even though they are often completely emptied before refilling, larvae have a good chance of surviving the short interim period in the water film which persists for some time even at the bottom of an up-ended container. This was found to be the case at Fakaofo where, before the survey of the village itself took place, many householders inverted their 44-gallon drums having gained the impression that they were to be reprimanded if the search disclosed active mosquito breeding on their premises!

In the copra-producing islets of Atafu, numerous briquettes were re-located in "tungu" and natural tree-holes and a few were also found in the rat-gnawed coconuts into which they had been inserted in 1958. All these coconuts proved to be rotten and far beyond the stage of holding liquid and serving as mosquito larval habitats. Of 50 "tungu" containing briquettes, 31 (62 per cent.) lacked larvae, as compared with a 100 per cent. larval incidence in 25 without briquettes.

Briquettes from larva-free containers were tested against freshly collected A. polynesiensis at both Atafu and Nukunono, and complete and rapid kills were registered. On the other hand, briquettes from tree-holes and the drum in which mosquitos were developing, failed to kill any of the larvae exposed to them at either atoll. From their irregular size and shape it is considered that all these non-toxic briquettes belonged to the first of the two batches manufactured at Atafu in 1958. These were of a rather granular appearance as compared with those of the second batch and it is considered that their dieldrin was released prematurely, if indeed the larvicide had been evenly distributed throughout the mix in the first place.

At Atafu, where rats were commoner than at the other two atolls and where a higher percentage of the mosquito population consequently originated from rat-gnawed coconuts, repetition of the 19 1958 adult catches gave a mean of 90.5 by comparison with the previous figure of 123.1 (see table, page 3). On certain islets of this atoll, "tungu" and tree-holes produce the bulk of the mosquitos because of a local scarcity of rats. Adult incidence there was reduced below the level of the over-all figure.

In July 1963, a New Zealand expedition to the Tokelaus obtained further follow-up information. On this occasion, A. polynesiensis larvae were collected from 35 container habitats at Nukunono. These specimens were sent to Professor J. N. Couch of the University of North Carolina, a leading authority on Coelomomyces, who recorded parasitized larvae[1] from 13 (37.1 percent.) of the samples - a fourfold increase over the 1960 figure. It was also found that even after almost five years, dieldrin-cement briquettes remained larvicidally effective at Atafu. Fifteen briquettes now recovered were sent by WHO to the Woodstock Agricultural Research Centre (Sittingbourne, Kent), for detailed study. Analysis by total chlorine and gas-liquid chromatographic methods subsequently established that briquettes from tree holes (which dry out from time to time) and those from (constantly replenished) domestic water drums had lost about 50 per cent. and 60 per cent. respectively, of the available dieldrin. It was thus concluded that the briquettes still had an extremely long useful life, with the reservation that at some stage leaching of dieldrin might cease (e.g. owing to the build-up of a coating of impervious foreign matter) despite the continuing presence of a fairly high concentration of the insecticide.

Then, in October-November 1963, a member of the New Zealand Department of Health visiting Atafu in another connexion, made fat biopsies from two volunteers from each of six households which had relied completely upon briquette-containing tanks for their

---

[1] Professor Couch also reported that in some instances a second species of Coelomomyces, and suspected hybrids between this species and the dominant C. stegomyiae, were present. This second species proved identical to one recently found by Professor Couch when examining specimens of C. stegomyiae-positive A. aegypti and A. albopictus from Rangoon, Burma (Muspratt, 1964), and it should be mentioned that such double infections are rather common with respect to Coelomomyces. It thus appears that two species of the genus instead of one were introduced into Nukunono in 1958, the new one referred to having escaped detection earlier through an overwhelming dominance of the larger sporangia of C. stegomyiae in the material originally derived from A. albopictus at Singapore.

water supplies over the preceding five years. No trace of dieldrin could be detected in any of these samples on analysis (sensitivity of method, 0.2 µg dieldrin) at the Wallaceville Animal Research Station of the N.Z. Department of Agriculture.

Finally, to profit from the latest refinements in relevant analytical techniques, arrangements were made for serum samples to be taken from 20 inhabitants of Atafu during a filariasis survey there in October 1965. Analyzed (in the Toxicology Section, CDC, PHS, United States Department of Health, Education and Welfare, Atlanta, Georgia) by electron capture gas chromatography, these exhibited an average dieldrin concentration of only 0.0010 ppm (range, <0.0001-0.0047). The average concentration was in fact little more than half that now found in the general population of the United States of America; and although the highest level recorded exceeded the relevant figure (0.0032 ppm) found by this laboratory in people in the general American population, the difference is not considered great enough to be of any significance to health. As the loss of dieldrin from tissues is very slow, particularly at low levels of storage, it is believed that the blood levels for Atafuans may have been little higher on the occasion of the 1963 sampling, after which few if any of the briquettes manufactured in 1958 were still present in local drinking water containers.

Besides yielding the information briefly presented herein, and thus demonstrating the feasibility of developing practical integrated mosquito control methodologies combining selective chemical procedures with novel biological measures, the Tokelau Islands project suggested a number of topics for allied research; and perhaps most importantly of all, its encouraging results were an important factor in the decision of WHO to initiate a widespread biological control research programme in 1961.

All the specific recommendations for further research relating to the Tokelau Islands project itself and made in the reports of those concerned, have since been implemented in the course of these broader programme activities. For example, the project drew attention to the need for a great deal more information on the life-history, geographical distribution and host range of Coelomomyces, and for appropriate procedures for the mass cultivation of these fungal pathogens, as pre-requisites to future large-scale field trials. A WHO consultant, Mr J. Muspratt, has since made field studies of high natural mortalities in Anopheles gambiae resulting from heavy infection with a strain of Coelomomyces indicus locally abundant in rainy-season pools near the Victoria Falls, Zambia (Muspratt, 1962); and subsequently, the Organization has been supporting an intensive laboratory investigation of this strain at the University of Bristol, England (Madelin, 1964, 1965). Throughout this period, too, in response to a request

made by WHO (Laird, 1962), a great deal of Coelomomyces material has been made available by collaborating investigators in various parts of the world. Many new host and locality records have been obtained, and Professor Couch, who has been studying these collections on the Organization's behalf, is currently working on a comprehensive review which will raise the number of known species of Coelomomyces from the 1961 figure of 24, to approximately 38. Also, WHO consultants have undertaken initial field studies of other candidate biological control agents suited for use in the reduction of A. polynesiensis incidence not only in the Tokelaus but elsewhere in its area of distribution too (for example at Aitutaki, Cook Islands, where preparatory baseline data on the local ecology of this mosquito have already been obtained - Tamashiro, 1964). These other agents include Neotropical craneflies of the genus Sigmatomera, the aquatic larvae of which prey upon larval Aedes (Stegomyia)in tree holes (a search for living examples with which to establish a laboratory colony was initiated in Brazil in 1964, and is still proceeding); and predators on rats, meriting introduction into certain of the South Pacific islands in the interests of reducing the availability of rat-gnawed coconuts as A. polynesiensis larval habitats (Laird, 1963; Uchida, 1966). It is anticipated that in due course these and other biological control research programme activities will lead to further WHO-supported field trials in the South Pacific.

REFERENCES

Laird, M. (1962) Coelomomyces fungi, an important group of mosquito parasites. WHO/EBL/1, WHO/Mal/334, WHO/Fil/42 (mimeographed, 4 pp)

Laird, M. (1963) Rats, coconuts, mosquitoes and filariasis. Symposium on Pacific Basin Biogeography, 10th Pacific Science Congress, Bishop Museum Press, Honolulu, pp 535-542

Madelin, M. F. (1964) Laboratory studies on the infection of Anopheles gambiae Giles by a species of Coelomomyces. WHO/EBL/17, WHO/Mal/438, WHO/Vector Control/64 (mimeographed, 23 pp)

Madelin, M. F. (1965) Further laboratory studies on a species of Coelomomyces which infects Anopheles gambiae Giles. WHO/EBL/52.65, WHO/Mal/530.65 (mimeographed, 22 pp)

Muspratt, J. (1962) Destruction of the larvae of Anopheles gambiae Giles by a Coelomomyces fungus. WHO/EBL/2, WHO/Vector Control/2 (mimeographed, 11 pp)

Muspratt, J. (1964) Parasitology of larval mosquitos, especially Culex pipiens fatigans Wied., at Rangoon, Burma. WHO/EBL/18, WHO/Vector Control/65 (mimeographed, 19 pp)

Tamashiro, M. (1964) Observations on the larval ecology of Aedes (Stegomyia) polynesiensis Marks on Aitutaki, Southern Cook Islands. WHO/EBL/22 (mimeographed, 29 pp)

Uchida, T. A. (1966) Observations on the monitor lizard, Varanus indicus (Daudin) as a rat control agent on Ifaluk, Western Caroline Islands. WHO/EBL/66.64 (mimeographed)

The purpose of the WHO/EBL series of documents is threefold:

(a) to acquaint WHO staff, national institutes and individual research or public health workers with new developments in the field of environmental biology;

(b) to distribute to the groups mentioned above field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;

(c) to make available to these groups some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

The mention of manufacturing companies or of their proprietary products does not imply that they are recommended or endorsed by the World Health Organization.