ESTABLISHMENT AND LONG-TERM SURVIVAL OF *ROMANOMERMIS CULICIVORAX* IN MOSQUITO HABITATS, TOKELAU ISLANDS

MARSHALL LAIRD[1], JOEL URDANG[2] AND IONA TINIELU[3]

ABSTRACT. Man-made treeholes ("tungu") hollowed out from the lower part of the trunk of standing coconut palms are a feature of the Tokelau Islands (tropical Polynesia). Rainwater trapped in them is near neutrality and of low electrical conductivity, and serves as an important permanent source of the Bancroftian filariasis vector, *Aedes polynesiensis*. From 2–5 June 1978 the population of this mosquito on the islet of Fenuafala, Fakaofo atoll, was exposed to imported *Romanomermis culicivorax* (eggs in sand cultures). A temporary establishment was confirmed in August 1978 and a monitoring visit in November 1980 revealed that the worm was still causing infections in at least 3 of the tungu initially treated. Subsequently (15 May 1981) these yielded additional parasitized larvae. The establishment had thus persisted for almost 3 years following a single inoculative application. Data presented suggest the future usefulness of *R. culicivorax* as an element in an integrated mosquito control methodology designed for the Tokelaus.

INTRODUCTION

New Zealand's Tokelau Islands dependency consists of 3 atolls (Fakaofo, Nukunono and Atafu) lying about 80 km apart, ca 500 km north of the Republic of Western Samoa, and ca 1000 km east of Tuvalu.

In 1958–60 the Tokelaus were the scene of parallel biocontrol/chemical larviciding experiments against the Bancroftian filariasis vector, *Aedes polynesiensis* Marks, using the fungal pathogen *Coelomomyces stegomyiae* Keilin at Nukunono and dieldrin/cement briquettes at Atafu. The project was supported by the World Health Organization (WHO) and the New Zealand Government (Laird 1967). Fakaofo served as the experimental control during the 1958–60 project. Relevant ecological data from the earlier project furnished a good basis for the present studies. Also supported by WHO, these featured the mosquito mermithid, *Romanomermis culicivorax* Ross and Smith.

It was desired to explore this worm's capacity for becoming established in the more permanent types of container habitats following a single inoculative application. While some experience elsewhere had indicated a need for repetitive, inundative-type applications, Washino (1981) and Platzer (1981) have reported on the ability of *R. culicivorax* to overwinter in Californian rice fields, suggesting the prospect of successful inoculative use.

It was hoped that the mermithid might help to solve the problem of *Ae. polynesiensis* production from tungu. These man-made, miniature reservoirs used to be adzed from the lower part (generally the underside, although not always so, Figs. 1, 2) of the bole of a standing coconut palm. Rainwater was channeled into the triangular (Fig. 3) or (occasionally) oval (Figs. 1, 2) opening by 2 grooves running down the trunk (Figs. 1–4). Outside of the Tokelaus, tungu only occur in parts of Tuvalu, as a culture-contact from the former group.

The volume of water stored by tungu clearly varies with rainfall. On the basis of estimates from ca 750 tungu (all atolls),

---

[1] Research Unit on Vector Pathology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7.

[2] University of South Carolina, Columbia, SC.

[3] Fenuafala Hospital, Fakaofo, Tokelau Islands, South Pacific.

Laird and Colless (1959) reported that 2–10 liters were usually present, while Hague (1868) mentioned "four or five gallons" (ca 18–23 liters). One exceptionally large tungu in a very old coconut palm was found (Laird and Colless 1959) to hold ca 100 liters. Especially in old tungu, the surrounding material is largely the long-dried outer cortex of the palm trunk. This precludes significant secretion from the tree itself into the stored water, the pH of which has been found to range from 6.0–7.6 at ca 26–27°C (Laird 1955). Tungu are thus very different from the usual kind of treeholes formed by natural rotting. The aqueous contents of such rot-holes tend to be discoloured and, to a varying extent, influenced by secretions from the tree itself. Chapman (1964) showed that, in California, treeholes in willows and cottonwoods, in which *Orthopodomyia* sp. and *Aedes sierrensis* (Ludlow) were present, had a pH range of 8.4–9.4 and 8.0–8.5 respectively; the salts-content being high in both cases, but appreciably higher in cottonwoods. Petersen and Chapman (1969) explored this point further for other types of treeholes, reporting the presence of various mosquito larvae at conductivities ranging as high as 2600 μs.

It is widely assumed that all treeholes are chemically comparable to the highly alkaline or acidic ones of e.g. northern temperate woodlands (Petersen and Chapman 1969). Since tungu waters are potable and near neutrality, it seemed worth examining their suitability for *R. culicivorax*, the optimal conductivity for which is near 100 μS, and maximum reported tolerance ca 1500 μS (Petersen and Chapman, personal communication 1978; Platzer 1981).

During the 1958–60 surveys, the total number of tungu visited at the 2 atolls completely surveyed were 486 (Nukunono) and 259 (Atafu), respectively. Most were old then, since the construction of tungu had virtually ceased years previously. Although no longer needed as a drinking water supply on each village islet where by 1958–60 cisterns and household water butts, made from 44-gallon (196 liter) oil drums, assured an adequate source except during drought or hurricane emergencies, tungu on the many outer reef islets were still used by fishing and copra-collecting parties. Filling tungu with sand and coral debris (Fig. 4) to prevent *Ae. polynesiensis* breeding was thus not generally practiced. Individual tungu endure for a very long time, and occasional trees survive for more than a century (Lambert 1970). Thus tungu are of decided importance as a permanent source of *Ae. polynesiensis*. Other larval habitats of this mosquito, aside from peridomestic water containers on each village islet, are relatively impermanent but renewable (rat-gnawed coconuts, dry shells, the bracts of fallen fronds, small natural treeholes which dry up intermittently, crab holes, etc.).

MATERIALS AND METHODS

Cultures of *R. culicivorax* were programmed to yield ca one million preparasites. The 1978 cultures were of infective material in the egg stage; those used in 1980 contained large numbers of adult mermithids. In both cases the cultures were supplied by the Gulf Coast Mosquito Research Laboratory. To minimize possible adverse effects on the worms by handling en route, the cultures (3 in 1978; 1 in 1980) were hand-carried throughout the journey to Apia as flight-cabin baggage.

Water temperature, pH and electrical conductivity were measured by means of a portable, battery-operated LaMotte Multirange Conductivity Meter.

RESULTS AND DISCUSSION

RELEVANT FIELD WORK (APRIL 1978) IN WESTERN SAMOA. From 22–30 April, ML worked in Samoa with Barry Engber, a Peace Corps worker (soon afterwards replaced by JU). Because transportation problems prevented a planned Tokelaus visit at this time, it was decided to use the opportunity for measuring the physical

characteristics of a variety of *Ae. polynesiensis* larval habitats, notably those types already known to be utilized by this mosquito in the Tokelaus, i.e., 44-gallon drums, treeholes in breadfruit trees *(Artocarpus communis)* and crab holes. A total of 78 sites was investigated, 20 of them on Manono Island and the remainder in various parts of Upolu (Satava and Falefa Villages, and Tia-Vi Lookout).

The conductivity of drinking water in 7 oil drums ranged from 8–100 (mean = 75) μS. The readings for 14 breadfruit ranged from 140–2200 (mean = 1000) μS. In addition, 5 crab holes ranged from 1600–9900 (mean = 3700) μS; *Ae. polynesiensis* larvae were present in all crab holes but one, which was close to the low-tide mark and held highly brackish water (9900 μS). While the salinity of strand crab holes seemed of too high electrical conductivity for *R. culicivorax*, the oil drum readings were optimal for this worm. Although treeholes in breadfruit trunks exhibited rather high conductivity, the mean fell within the worm's tolerance range and several readings were low enough (e.g. 140, 450, 660, 670 μS) to indicate suitability for *R. culicivorax*.

FIELD WORK ON FAKAOFO, 1978. From 2–5 June 1978, the second author (JU) attempted to establish *R. culicivorax* in 41 aedine larval sites on Fakaofo atoll at the village islet of Fakaofo itself, on the only other settled islet, Fenuafala, and on the very small islet of Teafua.

On Fakaofo, 5 treeholes were treated in breadfruit trees. In the village, nematodes were also added to 3 large rainwater tanks, 6 oil drums containing potable water, and a disused outrigger canoe.

On Teafua, 3 tungu were the only mosquito larval habitats treated. Preparasites of *R. culicivorax* were added on Fenuafala to: 2 large rainwater tanks, and 9 oil drums containing potable water; 7 natural treeholes in breadfruit and puka *(Hernandia ovigera)* trees; 3 tungu and one old canoe.

All the above sites were containers, usually the only types of mosquito larval habitats in the Tokelaus. However, Fenuafala and the Ahanga-Olopuka district of Atafu are unique in the group in having a number of pit-gardens, called "autu[4]." Two of these, up to 10 m^2 and 1.5 m deep, were investigated. Autu at Fenuafala had been found to harbor *Aedes vexans nocturnus* (Theobald) by Laird and Colless (1959), while Urdang collected 2 larvae there subsequently identified as *Culex quinquefasciatus* Say by the Smithsonian Institution, Washington, D.C.

The infective material applied on Fakaofo was derived from 2 *R. culicivorax* cultures. Immediately before leaving Apia for the 36-hour voyage to Fakaofo, 3 × 3 cc samples were taken from each of the 3 damp-sand cultures brought by ML in April. The 9 samples were placed in individual plastic vials, each containing 100 ml water and left to stand overnight. Next morning, all samples were examined for active preparasites. Ten aliquots of 0.1 ml each were taken from each of the 9 samples and examined for

---

[4] Autu are fertilized pits dug several meters into the ground for the cultivation of pulaka, or taro *(Colocasia)* and sometimes other food plants, including bananas. Each autu is surrounded by a shallow ditch at the bottom of the pit, and pockets of rainwater collect there.

Fig. 1 (upper left). Tungu of oval type, arrows indicating rainwater entry grooves—LaMotte Multirange Conductivity Meter in use; Fig. 2 (upper right). Tungu of oval type, arrows indicating one of the two rainwater channels around the palm trunk; Fig. 3 (lower left). Commonest, triangular form of tungu, showing entry grooves and crab (*Sesarma gardineri* Borradaile), which is often present in tungu; Fig. 4 (lower right). Tungu blocked with sand and coral, to prevent use by *Aedes polynesiensis*. The rainwater entry groove at right has been blocked by ants.

worms under a dissecting microscope, the number of preparasitic nematodes per 3 cc sample being calculated as: culture 1, 1566; culture 2, 1133; and culture 3, 1933. Culture 1 was used on both Fenuafala and Teafua islets; culture 2 was used on the main village islet of Fakaofo; and culture 3 was retained in Apia.

Varying amounts of the infective material were spooned into larval habitats, ranging from 0.4 cc in tungu on Fenuafala (up to 25 *Ae. polynesiensis* larvae per site) to 60 cc in a Fenuafala autu (containing "thousands" of *Ae. vexans nocturnus* larvae). Forty-one larval habitats were treated; the goal being to introduce ca 10–15 preparasites per mosquito larva.

Sampling before leaving Fakaofo indicated that mermithids were evident in 3.2% of 500 *Ae. polynesiensis* larvae collected from treeholes and tungu with a range of 490–3400 (mean 980) μS conductivity (upper readings believed to be 10× too high and mean therefore probably inflated). The worms were also present in 32% of 877 *Ae. aegypti* (Linn.) larvae, mostly collected from domestic water drums with conductivities of 21–110 (mean = 49) μS. *Aedes aegypti,* an important vector of hemorrhagic dengue, was previously unknown in the Tokelaus, its accidental importation having apparently taken place in the late-1960's (Urdang and Pillai 1978, Pillai and Urdang 1979).

A return trip 2 months later enabled JU to confirm the presence of *Ae. aegypti* on Nukunono and Atafu atolls as well as Fakaofo. While revisiting the latter, he sampled 25 of the 41 sites earlier treated. Only 6 (24%) failed to yield parasitized larvae; 4 of these were considered "questionable." Of 289 larval *Ae. aegypti* collected from 11 sites, 64 (22.1%) exhibited *R. culicivorax*, while 52 (18.5%) more were listed as "questionable." Of 249 *Ae. polynesiensis* larvae collected, 34 (13.6%) contained *R. culicivorax* and 76 (30.5%) were considered "questionable."

A high incidence of "questionables" occurred because, due to a shortage of time, the larval samples had to be preserved (Macgregor's solution)and transported back to the laboratory in Apia for microscopic examination; under such circumstances it is often difficult to be absolutely certain whether or not small worms are present.

The initial application had been made 9 weeks earlier. As one full generation of *R. culicivorax* requires approximately 7 weeks, at least a temporary establishment had thus been achieved. Parasitized *Ae. aegypti* larvae were recovered from 12 of 14 oil drums sampled (the other 2 were listed as "questionable"), and from one of 3 large metal tanks (the 2 negatives were based on samples of only 2 and 3 larvae respectively).

Parasitized *Ae. polynesiensis* accompanied parasitized *Ae. aegypti* in only one of the oil drums, although 6 others also yielded *Ae. polynesiensis*—in 2 drums listed as "questionable." Parasitized *Ae. polynesiensis* were collected from one of the 3 tungu and 3 of the 7 natural treeholes earlier treated in Fenuafala, where an additional treehole contained 14 larvae, all of which were listed as "questionable."

The third author (IT) made some collections in between JU's 2 visits. Thus, on 23 June and again on 7, 13 and 19 July, he found parasitized larvae (presumably *Ae. polynesiensis*) in tungu, also in a treehole which was visited on 23 June and 7 July. In addition, 2 large water tanks and 4 oil drums yielded positive larvae which may have been *Ae. aegypti* or *Ae. polynesiensis.*

RELEVANT EVENTS IN FAKAOFO, 1979. March and April 1979 proved unusually dry at Fakaofo. Drought conditions prevailed and all water drums about the village dried up completely. Moreover, some treeholes and tungu were deliberately blocked with sand and coral blocks during this period (Fig. 4) in an attempt to control mosquitoes following a dengue outbreak. IT found the smaller treeholes also dried out and the remaining tungu became damp-dry. In May, after some rain had fallen, IT arranged for the collection of mosquito larvae from 7 oil drums, one water tank, 3 tungu, 2 treeholes and 1 autu. These larvae, preserved in Macgregor's solution, were shipped to JU in Apia. They consisted of 302 *Ae. polynesiensis*, 262 *Ae. aegypti* (from the metallic containers), and 178 *Ae. vexans nocturnus* (from the autu). None yielded any trace of nematode parasitism. the autu). None yielded any trace of nematode parasitism.

FIELD WORK ON FAKAOFO, 1 NOVEMBER 1980. ML spent 1 November with IT on Fakaofo. The man-made containers comprising the bulk of the sites treated in and around the main village on Fakaofo were not examined, as all of these had dried out completely for several weeks during the previous year's drought. Time ashore was short so ML's survey was confined to Fenuafala. Three of JU's sites held *Ae. polynesiensis* larvae parasitized by *R. culicivorax*. One of his tungu (Site 4) yielded 15 uninfected larvae. Of the positive sites, 2 were tungu, the first with five 2nd- to 4th-instar larvae of which one was parasitized, and the other from which 15 larvae were taken; again one was parasitized. Two of 25 larvae from JU's natural treehole in a coconut trunk were infected also.

Three larvae from the last-mentioned site had proved uninfected on examination 4 days after the original 1978 application. The first of the 2 positive tungu sampled yielded 123 larvae at that time, 119 being uninfected and 4 (3.4%) possibly so; while of 19 larvae then taken from the other tungu, 16 were uninfected, one (5.9%) positive and 2 (11.8%) possibly so. None of these 3 sites were sampled in August 1978 because of time constraints. With further regard to the second tungu, IT had observed 7 parasitized larvae there on 16 and 23 June, 8 on 7 July, 7 on 13 July, and 5 on 19 July 1978. Each successive sampling, of course, removed a proportion of the worms potentially capable of perpetuating the establishment.

The conductivity of the first tungu was recorded by JU as 560 μS. ML's reading for 1 November was 340 μS. The early July 1978 reading for the natural treehole site was 1200 μS; ML's was 1400 μS. JU's conductivity reading for the second tungu was 340 μS, while ML's was 640 μS. Three additional tungu were examined at Fenuafala by ML and IT on 1 November. These were marked as having been visited by JU and IT in 1978, but no *R. culicivorax* had been introduced into them because they were dry at the time. The 3 tungu held water in November. They yielded 2, 6 and 8 *Ae. polynesiensis* larvae, all negative for mermithids. The conductivity of these tungu was 1400, 1100 and 380 μS respectively; the pH ranged from 6.8–7.2; and the water temperature from 21–22°C.

Material from the 1980 culture of *R. culicivorax* adults was spooned into the 3 parasite-free tungu. The remainder of this culture was left with IT for treating the autu once night-biting *Ae. vexans nocturnus* (and *Cx. quinquefasciatus?*) became troublesome.

FIELD WORK ON FAKAOFO, 6 NOVEMBER 1980–15 MAY 1981. The tungu from which 1 of 15 *Ae. polynesiensis* was positive on 1 November was re-examined by IT on 6 November 1980 and again on 14 January and 15 May 1981. Two mermithids were noted on the first date, one on the second and 2 on the third. The second tungu was positive on 1 November and again (numbers not recorded) on 6 November and 18 February. The incidence of parasitism was not measured in the field on either occasion, but 6 (11.5%) of 52 larvae parasitized on 18 February and forwarded to the Research Unit on Vector Pathology proved positive when examined by Dr. J. R. Finney.

On 17 December 1980 and again on 14 January 1981, a single infection was recorded from one of the previously mermithid-free tungu treated on 1 November. A second proved similarly positive on 14 January. These tungu were also sampled during March and April 1981 without positive findings.

During November 1980, the *R. culicivorax* culture left with IT was duly applied to the autu as night-biting mosquitoes became plentiful. As a purely subjective observation, IT recorded that the post-application incidence of night-biting mosquitoes in Fenuafala's inhabited area was much less than observed previously during the same season.

CONCLUSIONS

Although the 1979 drought disrupted the *R. culicivorax* experiments in peridomestic container habitats in the village area of Fenuafala, the fact that infections in *Ae. polynesiensis* in "bush" tungu have persisted for at least 35 months following a single introduction of eggs of *R. culicivorax* from a damp-sand culture, establishes that the inoculative use of this mermithid might be practical under such conditions as part of an integrated control program directed against the Bancroftian filariasis vector, *Ae. polynesiensis*.

ACKNOWLEDGMENTS

Warm appreciation is expressed to the Faipule (= Head of Council) and the Council of Elders, Fakaofo, for consenting to these experiments there. Support from WHO, the New Zealand Government, and the Office for Tokelau Affairs, Apia, is acknowledged with gratitude, as is the indispensable aid of Gulf Coast Mosquito Research, Laboratory, U.S. Department of Agriculture, Lake Charles, Louisiana, in providing the mermithid cultures used in 1978 and 1980.

References Cited

Chapman, H. C. 1964. Observations on the biology and ecology of *Orthopodomyia californica* Bohart (Diptera: Culicidae). Mosq. News 24:432–39.

Hague, J. P. 1868. Some coral islands and islanders. Atlantic Monthly 7:36–51.

Laird, M. 1955. Notes on the mosquitoes of the Gilbert, Ellice and Tokelau Islands, and on filariasis in the latter group. Bull. Entomol. Res. 46:291–300.

Laird, M. 1967. A coral island experiment: a new approach to mosquito control. WHO Chron. 21:18–26.

Laird, M. and D. H. Colless. 1959. Experiments towards the biological control of mosquitoes in the Tokelau Islands. First Report. MHO/PA/93.59 WHO (Mimeographed).

Lambert, M. (Ed.). 1970. Coconut production in the South Pacific. SPC Handbook No. 6. South Pacific Commission, Noumea, New Caledonia.

Petersen, J. J. and H. C. Chapman. 1969. Chemical factors of water in tree holes and related breeding of mosquitoes. Mosq. News 29:29–36.

Pillai, J. S. and J. Urdang. 1979. The discovery of the mosquito *Aedes aegypti* on Tokelau group. N.Z. Med. J. 90:212–13.

Platzer, E. G. 1981. Biological control of mosquitoes with mermithids. J. Nematology 13:257–62.

Urdang, J. and J. S. Pillai. 1978. Recovery of *Aedes aegypti* from Tokelau. So. Pacific Comm. Dengue Newslett. 6:9.

Washino, R. K. 1981. Biocontrol of mosquitoes associated with California rice fields: with special reference to the recycling of *Lagenidium giganteum* Couch and other microbial agents, pp. 122–39. *In* Biocontrol of medical and veterinary pests. (Ed. M. Laird). Praeger, New York.