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Review

THE PRESENT STATUS AND FUTURE PROSPECTS OF MALARIA CONTROL

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INTRODUCTION

Malaria remains a major public health problem in most of the almost 100 countries or territories in which it is still endemic. It has been estimated that the number of new clinical cases is on the order of 100 million annually (WHO, 1987). While in many of the endemic countries the epidemiological situation has remained static, in others it has worsened and there have been a number of countries in which a serious recrudescence has occurred within the last few years, among them Sri Lanka and Brazil. There are many different reasons for this complex situation and some of them will be touched upon below, following a general review of the current epidemiological situation.

THE GLOBAL MALARIA SITUATION

Of a total world population of some 5,000 million people, more than 2,000 million live in areas where malaria has either never existed, has disappeared spontaneously following social and economic development, has been eradicated or where the risk of infection has been eliminated through improved health and economic conditions.

In areas where some 2,500 million people live (50% of the world's population), efforts are being made to reduce or control transmission through the application of antimalaria measures of one type or another; these include the majority of malarious countries of Asia and the Americas. However, the health services in many of the countries of these regions are not sufficiently developed to maintain the reduced level of transmission that some of them were able to achieve through concerted malaria control programmes and as many are now faced by economic and social difficulties, their malaria situation may grow worse and transmission periodically intensifies.

In the remaining malaria endemic areas of the world where more than 400 million people live, no specific measures are undertaken to control the transmission of malaria and, in these areas (for the most part Africa south of the Sahara), the prevalence of malaria remains unchanged.

The following section will review the malaria situation by region and sub-region.

AFRICA NORTH OF THE SAHARA

The total population of this sub-region is over 100 million people and almost 80 million of them live in areas that were at one time malarious. Libya and Tunisia have only reported imported cases of malaria while but a small number of indigenous cases now occur in Algeria. Malaria is of low intensity in Egypt and transmission occurs only in limited areas. In Morocco, however, transmission has increased in recent years. The malaria control programme is attempting to deal with this increase which in any event is limited in nature and the total number of cases annually is less than a thousand.

AFRICA SOUTH OF THE SAHARA

Of the more than 450 million people who live in this region, only 53 million live in areas where malaria has either never existed, such as in most of South Africa and Lesotho, or in those limited areas from where it has been eradicated. In the remaining areas, however, the endemicity of malaria has remained virtually unchanged over the years.

While most countries of this region had developed strategies for the control of malaria in the past, a reduction of malaria prevalence, especially in their rural areas, is not feasible. The reasons are technical, operational, administrative and financial; little, in fact, can be done other than reduce mortality and morbidity through the improvement of diagnostic and treatment services and through the more appropriate use of antimalaria drugs, especially in limited target groups such as pregnant women and children. Even this restrained objective is often difficult to meet due to shortages of drugs and to the poorly developed primary health care systems which can not always ensure the prompt diagnosis and treatment of clinical malaria; the spread of chloroquine resistant strains of *Plasmodium falciparum* in Africa has made therapy more difficult. Nevertheless, there are some areas where mortality from malaria alone is lower today than was previously the case, even among infants (Carnavale and Vaugelade, 1987); this change has been attributed principally to the easy availability of the antimalaria drugs (WHO, 1986a) which at least commercially, are widely available. Unfortunately antimalarial drugs are very frequently used incorrectly by populations who take inadequate dosages of the drugs for periods of time too short to achieve a radical cure. As noted above, the appearance of resistance may also reduce their broad efficacy.

There are certain situations usually associated with major ecological or socio-economic changes which have resulted in a focal worsening of the situation; an example of such a situation is the displacement of populations with limited levels of immunity into areas of high transmission that occurred as a result of the Sahel drought. Currently a very severe malaria epidemic is taking place on the plateau region of Madagascar due primarily, apparently, to a poor economic situation which badly hampered the continuing implementation of malaria control measures. Transmission on the plateau is by *Anopheles funestus* which has reinvaded the high plateau region of the island after being absent from the area for many years (Fontenille and Rakotoarivony, 1988).

Broadly speaking, vector control is of limited efficacy in this region. The principal vector species freely feed and rest both indoors and outdoors and thus the application of residual insecticides to the interior of houses has only a limited effect on the transmission of malaria despite the high level control which is frequently achieved of house entering mosquitoes.

While indoor resting densities may be reduced, malaria transmission is likely to continue at much the same rate. There are also many other difficulties associated with vector control including the problem and great cost of achieving coverage of all the dwellings in the endemic rural and peri-urban areas and the focal occurrence of insecticide resistance. Nevertheless considerable interest is being expressed in the use of insecticide impregnated bednets, curtains and screens which may at least reduce the frequency of infections by malaria. Insecticide resistance to the chlorinated hydrocarbon insecticides DDT, dieldrin and HCH is widespread and the limited number of urban and peri-urban vector control programmes making use of residual insecticides must apply OP, carbamate or pyrethroids whose cost is considerably greater than that of the chlorinated hydrocarbons such as DDT. In a number of countries efforts are being made to at least improve the control of malaria in peri-urban areas and, as an example, a Japanese sponsored urban malaria control programme in Tanzania will make use of the OP insecticide fenitrothion for indoor residual applications along with other methods against the malaria vectors in Dar-es-Salaam and Tanga.

One of the most serious constraints to effective malaria control in the Africa region is the lack of trained personnel for the planning, organization, implementation, monitoring and evaluation of malaria control programmes. This shortage exists at every level, whether for personnel to carry out epidemiological or entomological surveillance, do research, clinical management of cases or the administration and supervision of control operations. The training of personnel should, therefore, have a high priority. A number of international training courses on basic malariology and malaria control have recently been sponsored by donor governments, among them Italy, France and the USSR as well as by international organizations such as the WHO and UNDP and other such courses are planned for the future.

The UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases has supported MSc courses in medical entomology and vector control in three centres in Africa in Bouake, Cote d'Ivoire, Jos, Nigeria and Nairobi, Kenya as relatively few African scientists have been previously trained in this discipline.

THE AMERICAS

It is estimated that the population at risk of contracting malaria in the America region has increased from 143.6 (30%) million in 1960 to 263.4 million (39%) in 1986. 950,471 cases of malaria were registered in the region in 1986 which was an increase of 57,236 cases over 1985 and the highest figure registered since 1958. Morbidity per 100,000 population in the malaria endemic areas rose from 164.95 in 1985 to 360.89 in 1986; almost 20 million people in the Region received no protection at all against malaria; it is of concern to note that the two most frequent reasons for such a lack of protective programmes were lack of resources in 58.1% of the cases and socio-political problems in 14.9% (PAHO, 1988).

The increase occurred in 12 countries in which control activities are being carried out i.e. Argentina, Bolivia, Brazil, Colombia, Costa Rica, Dominican Republic, French Guiana, Guyana, Mexico, Nicaragua, Panama and Peru.

In Mexico which reported 13.8% of all the cases in the Region, there was a 14.4% increase over the number in 1985. Fortunately, the proportion of cases of *P. falciparum* fell by 30% during the same period.

The malaria problem in Brazil which has 46.7% of the cases in the Region is associated

with a great human migration into the Amazon region of the country which in itself accounts for 96.3% of the cases of the disease in Brazil; 70% of these cases were registered in the two states of Para and Rondonia. Considerable efforts are underway to control the transmission of the disease in this geographical area including insecticide application and selective chemotherapy. The problem of controlling malaria in the Amazon and in forest areas contiguous to areas of human settlement is common to several countries in this part of South America.

The human population covered by intradomiciliary spraying in the Americas is some 25 and a half million. Despite the spread of DDT resistance in *Anopheles* species, especially in populations of *An. albimanus*, DDT still represents 89.3% of the insecticides applied, while fenitrothion is 5.1%, propoxur 2.0%, bendiocarb 1.0%, deltamethrin 0.6% and malathion and all others together 2.0%. Field trials of new insecticides continue in the Region to ensure the continuing availability of effective compounds for use where the development of vector resistance necessitates change to an alternative compound. As will be shown later, contamination of mosquito larval habitats by the agricultural use of insecticides both in the Americas and in other rural areas where malaria is present, is a major factor in selecting for insecticide resistance in mosquito populations even before the compounds have been used in public health.

The extent of development of *P. falciparum* strains resistant to chloroquine is very serious problem in South America and particularly in Brazil and Colombia. The necessity to use more expensive, alternative drugs in the areas where the use of chloroquine is to a great extent precluded adds to the cost of malaria treatment.

The situation in Guyana is serious; the index of parasitemia for the population of the malarious areas was 20.6 per thousand in 1986, the highest for the Region and, even more seriously, 56.6% of the infections were attributed to *P. falciparum*.

By contrast the number of cases of malaria in Guatemala has dropped substantially. Ninety-seven per cent of the cases in the country are due to *P. vivax* with most of the cases occurring in the northern part of the country. An even greater drop in the number of new malaria cases has been registered in Haiti where the number of new cases in 1984 was 69,863 and had fallen to 14,363 by 1986. The present status of control operations in the country is uncertain due to economic difficulties.

ASIA WEST OF INDIA

The total population of this region is 222 million people of which 186 million live in areas which were originally malarious. Fifteen million live in areas which have been freed from the disease, 35 million in areas of limited risk and some 4 million in areas where there are no antimalaria measures being carried out. Despite a high number of imported cases, Bahrain, Cyprus, Israel, Kuwait, Lebanon and Qatar all continue to be free from indigenous malaria.

In 1986, increases in malaria were recorded from Afghanistan, Iran, Pakistan, Yemen and Democratic Yemen, and the United Arab Emirates. The number of cases reported from Iraq, Syria and Saudi Arabia declined somewhat from the previous year. The malaria control situation in Afghanistan has deteriorated greatly and the number of cases reported may probably be an underestimate. One hundred and fifty-six thousand cases were reported in 1984 and more than 228,000 in 1985.

Malaria has traditionally been a major public health problem in what is now Pakistan and is still an important problem in part of Pakistan's population of 97.5 million. Epidemics frequently occur in the irrigated plains of the Punjab and Sind which are the most densely populated parts of the country. While most of the population of the country and most of the malaria transmission is rural, persistent urban malaria transmission in some urban areas has necessitated that malaria control be carried out in the cities as well. The main rural vector is *An. culicifacies* and in the urban areas it is *An. stephensi*.

A malaria eradication programme was started in Pakistan in 1960 with a good deal of initial success. However, the poor participation of the general health services, a reliance on house spraying as the sole control measure, the development of resistance to DDT and HCH, and economic difficulties led to a very serious recrudescence of the disease in the early 1970s with an estimated 10 million cases having occurred in 1972 (AID Report, 1987) (though the official figure was but 642,958 microscopically confirmed cases; EMRO, 1987). A number of improvements were introduced into the programme in 1975 including the replacement of the organochlorine insecticides by malathion, the inclusion of urban areas in control and various administrative changes. While the situation improved until 1980, many difficulties are still facing the programme and the number of cases rose from 17,707 in 1980 to the 90,393 cases reported in 1986 (AID Report, 1987). Furthermore chloroquine resistant *P. falciparum* strains have also appeared in Pakistan. Even more seriously the proportion of *P. falciparum* has risen from 13.32% to 33.39% of the total positive slides (Report of External Evaluation Group, 1985).

While it had been thought that the presence of large numbers of Afghan refugees in Pakistan lead to a worsening of the malaria situation by bringing a heavy load of malaria with them, investigation showed that in actuality the refugees themselves faced a high risk of malaria infection on their arrival in Pakistan (Suleman, 1988).

THE INDIAN SUBCONTINENT AND ISLANDS OF THE INDIAN OCEAN

Of the total population of 884 million people in this area some 96% or 850 million are exposed to malaria risk to varying degrees. Most of the original malarious areas must still remain under some type or another of malaria control.

Numerous problems exist in the region of a technical, financial, operational and managerial nature. Resistance to insecticides in vector species and resistance to drugs of *P. falciparum* are both wide spread; resistance to fansidar has also appeared in Bangladesh. As regards the number of reported cases, there has not been a serious general worsening of the situation in any area other than in Nepal in the last year or two; however, on the other hand, relatively little progress is being made and the overall situation in the region appears to have stabilized at the existing level of transmission. But in view of the spread of drug resistant strains of *P. falciparum* and the continued spread of resistance to insecticides in important species of vectors, such a stabilization cannot be considered a satisfactory situation.

In Bangladesh, the entire population of the country lives in areas where malaria transmission occurs; residual insecticide applications are carried out in areas where 2.3 million people live. Sixty per cent of all malaria cases and 93% of all *P. falciparum* cases are now reported from districts in the eastern border area of the country. Though the number of reported cases of malaria remains more or less the same, the proportion of the cases due to *P. falciparum* has

risen from 40% in 1983 to 45% in 1984 and 49% in 1985. Although the number of individual reports of chloroquine resistance is rising, most of them are still restricted to forested areas of the east and north-east of the country.

Malaria transmission in Bhutan has stabilized or declined somewhat but the chloroquine-resistant strains of *P. falciparum* apparently imported from India have established local transmission of such strains in the country.

Of the population of more than 750 million people in India, at least 728 million people live in areas where malaria transmission continues and at least half this number live in areas of high transmission. Some 100 million people are covered by the *P. falciparum* Containment Programme which is itself part of the National Malaria Eradication Programme. More than 63 million people in urban areas are covered by programmes based essentially on antilarval measures. Urban malaria transmission remains a serious problem as the larviciding on which it is based does very little to permanently ameliorate the transmission by *An. stephensi* (Hyma *et al.*, 1984). Sharma and Mehrotra (1986) believe that the resurgence of malaria in India preceded the true problem of insecticide resistance and that the malaria resurgence occurred in towns where control measures were non-insecticidal and in regions which were not under the influence of transmission by insecticide-resistant vectors.

Throughout the country the number of new cases every year has stabilized at a reported figure of about 2 million and the increase in percentage of cases due to *P. falciparum* seems to have peaked at 29% in 1984 as compared to 9.7% in 1977 (WHO, 1987). On the other hand resistance to chloroquine continues to spread as does *Anopheles* resistance to insecticides. Resistance to the organochlorines and, increasingly, to malathion, continues to intensify among populations of *An. culicifacies* and both *An. stephensi* and *An. annularis* have shown resistance to DDT and HCH. In some areas the level of resistance to DDT, HCH and, to a lesser extent, malathion in *An. culicifacies* populations is high enough to prevent interruption of transmission by use of these insecticides (Vittal *et al.*, 1982). This necessitates a continued search for new compounds that can be used as replacements for those to which resistance has developed for use in indoor residual spray programmes. The alternative would be to abandon residual spray programmes but this might well have serious consequences in a number of areas.

The number of new cases of malaria in Nepal has been steadily rising from 17,000 cases in 1983 to 42,000 cases in 1985. Economic constraints are a problem in the purchase of insecticides and part of the country remains without any antimalaria protection at all. The malaria programme already consumes 20% of the national health budget (Najera, J.A., personal communication).

Out of a population of about 16 million people in Sri Lanka, at least 12 million live in areas where malaria transmission continues. The malaria control situation in the country has continued to deteriorate for several reasons. Part of this is obviously due to the political situation which renders control activities difficult to implement in certain areas. However, in very many areas the problem involves a poor level of management and operational supervision of the programme along with a lack of supplies including transport and petrol. The difficulties are increased by the rapid spread of resistance to DDT in the only vector, *An. culicifacies* and the appearance and spread of resistance to malathion despite a considerable effort which had been made to avert it. As control depends on insecticide coverage in areas where some 4 million people live, this is a serious development. Moreover, after several years

of an increase in the proportion of cases due to *P. falciparum* (from 2% of the cases in 1982 to 20% in 1986, Samarasinghe, 1986), chloroquine resistant strains of the parasite have appeared and have become well established. Improvement is badly needed in the management and supervision of the programme lest the numbers of cases begin to rise even more steeply as has occurred in the past in Sri Lanka. As it is, the number of new cases increased from 38,566 in 1982 to 149,470 in 1984, decreased to 117,816 in 1985 and, in 1986, has risen to almost 420,000. Furthermore, malaria is appearing in new areas of the country, around the city of Kandy specifically, and the incidence is increasing in the areas of water development schemes such as in the Mahaweli development area (Wiejesundera, 1988). As water development schemes grown in number and in area, the spread of malaria as a result could become a major threat (2nd Independent Assessment of the Intensive Malaria Control Programme, Anti-malaria Campaign, 1984).

EASTERN ASIA, THE WESTERN PACIFIC AND OCEANIA

In this vast area of 1,670 million people, 1,324 million live in originally malarious areas. Malaria has been eradicated from areas where 244 million live and the risk is considered limited in areas with a population of 531 million.

Australia, Brunei Darussalam, North and South Korea, Hong Kong, Japan, Macao, Mongolia, Singapore and large areas of China are considered to be free of malaria (WHO, 1987).

The number of reported cases in China has been steadily declining and is now on the order of 500,000 a year. The well supervised and intensive control work being carried out, integrated with the health services, should reduce the endemicity even further. One of the problem areas which remains is Hainan Island where the malaria is endemic in the mountainous and hilly areas. Chloroquine resistance in *P. falciparum* is a problem mainly in Hainan Island, and in some focal areas of the south of China.

Malaria is considered the leading public health problem in Burma and has been accorded the top priority in the national health plan for 1986-1990. Out of an estimated population of some 36 million, 22.1% live in areas where control is based on the government making antimalarial drugs available, 13.6% in areas covered by indoor residual spraying with DDT, 41.9% in surveillance areas, 13.9% in vigilance areas and 8.5% in non-malarious areas (Kondrashin, 1986). The resistance of *P. falciparum* to chloroquine is a serious and widespread problem in the country. Resistance has also been found to sulfadoxine/pyrimethamine and has been spreading rapidly. Among the vectors, *An. annularis* is resistant to DDT in the north-west part of the country where it is a vector in the coastal areas, and there are some reports of resistance from other areas and from other species. No alternative insecticides are in operational use or under field trials in Burma.

The programme in Burma is faced by many difficulties including difficult access to many border areas, a shortage of insecticide and transport and the still growing problem of drug resistance.

A population of some 46 to 47 million people in Thailand is considered at some risk to malaria. Of this number some 11 million live in areas where they are protected by control operations and 35 million in areas where malaria transmission has been reported as having been interrupted. In most of the latter area, the malaria services have now been integrated

with the general health services. Three million people live in areas where indoor residual insecticide spraying continues to be applied. The incidence of malaria has been greatly reduced throughout the country in the last three decades though some serious problems persist such as the increase in the proportion of *P. falciparum* from the early 1980s and a spread of drug resistance in this species. The control of exophilic vectors *An. minimus*, *An. balabacensis* and *An. dirus* in the forested, mountainous parts of the country is a problem for the control organization as is the control of malaria in and around the refugee camps in the border areas with Kampuchea and Vietnam. Most of areas covered by residual spraying still make use of DDT though large areas have been sprayed by fenitrothion as well.

Malaria is well controlled in Peninsular Malaysia and Sarawak with only some 10,000 and 1,000 cases a year respectively recently recorded. More than 90% of the total population live in areas freed from malaria transmission and most of the remaining transmission is in hilly, undeveloped areas on the peninsula and in the border areas of Sarawak. In Sabah, where most of the cases are caused by *P. falciparum*, resistance to chloroquine is widespread and the disease remains a serious problem; the number of cases has recently increased considerably. Integrated malaria control is carried out by the health services by spraying, drug distribution and increased surveillance as appropriate to the various endemic areas.

Some years ago, it was estimated that 83% of the total area of Kampuchea was malarious and that 35% of the total population were living in malarious areas (Harinasuta *et al.*, 1982). Because of the political situation within the country, little accurate information is available on the incidence of malaria. Antimalaria measures have begun again recently though of a limited nature. Transmission will probably continue within Kampuchea until a greater degree of stability is achieved in the country.

In the refugee camps on and near the Thai-Kampuchean border malaria is reported as the most serious communicable disease problem (Meek, 1988), especially where inhabitants are exposed to *An. dirus* populations in forested areas. Most of the transmission is due to *P. falciparum* especially in those camps with the most malaria transmission; the strains of *P. falciparum* in this areas are, for the most part, also resistant to chloroquine and fansidar.

Malaria also remains a major public health problem in the Lao People's Democratic Republic and antimalaria activities are very limited in scope, probably only to insecticide spraying in certain areas. The parasite rate for the entire country is estimated at 10-12% (WHO, 1987) and most of the country's population of 3.2 million is at risk. Most of the transmission is of *P. falciparum* and chloroquine resistance showing RI and RII response occurs.

Malaria is one of the principal public health problems of Vietnam; while a satisfactory level of control has been achieved in the north, many difficulties still remain in the southern part of the country and in the mountainous areas of the centre. DDT spraying is widespread in the southern part of the country but the control programme faces many problems among them drug resistant strains of parasites, exophilic species of vectors and human population migrations.

One hundred and fifty-six million people of the total population of more than 163 million of Indonesia, live in malarious areas. Regular malaria control activities cover much of Java and Bali with a population of 94 million people and certain settlement areas at particular risk; the national budget for malaria control represents a high percentage of the total budget for communicable disease control. Indoor residual spraying is still an important component of

the programme and 5 million people live in areas where insecticide spraying is carried out on Java and Bali; there is also a limited amount of residual spraying on some of the outer islands. Focal larviciding is carried out against *An. sondaicus* in some of the lagoons in which it breeds particularly on Bali and, to a lesser extent, on the south coast of Central Java.

Most of the reported cases of malaria originate from Central Java; *An. aconitus* is the main vector in this area and is highly resistant to DDT and HCH, creating a serious problem for its control; DDT resistance has also been recorded from *An. sondaicus* in the same area. Large scale field trials with fenitrothion in Central Java showed that this compound would provide satisfactory control of transmission (Sudomo *et al.*, 1985). A large reservoir of malaria exists in the outer islands where control programmes are still limited in extent though an effort is being made to improve surveillance and treatment through primary health care programmes and health centres. Chloroquine resistant strains of *P. falciparum* have been detected in 22 of the country's 27 provinces.

The malaria situation has not changed in recent years in Papua New Guinea. The disease remains mesoendemic to hyperendemic in most of the originally malarious areas and is a serious public health problem. This has been compounded by shift from a predominance of *P. vivax* to that of *P. falciparum*. Furthermore chloroquine resistance strains of *P. falciparum* have also appeared. The effectiveness of vector control programmes has decreased and all of this has made the problems associated with the intense year-round transmission more severe (Cattani *et al.*, 1986).

The malaria problem in the Solomon Islands has been brought under control by an intensification of routine antimalaria control measures, including improved treatment of cases, better supervision of spraying activities and the strengthening of the rural health infrastructure. Special attention has been given to training activities and a new centre has just been opened with the support of the Government of Japan. A number of important problems persist including the high frequency of chloroquine resistant strains of *P. falciparum* and problems with control of the vectors.

The Philippines have had an intensive malaria control programme for a long period of time. While a considerable reduction in the intensity of malaria transmission was achieved in the early years of the programme, the incidence still remains quite in many parts of the country especially in those areas of difficult access and there has been little recent improvement made in the programme. Limited resources pose a problem for further expansion of activities even as part of the health services. The wide distribution of *P. falciparum* strains resistant to chloroquine adds to the other problems of control.

EUROPE

The only area of the WHO European region where malaria transmission still persists is Turkey. Though the malaria eradication campaign was quite successful at the onset, the cessation of much of the control activities and the reduction in surveillance coupled with greatly increased irrigation programme in the south eastern plains resulted in a major upsurge in the number of cases of the disease. The problem on the Cukurova plain where *An. sacharovi* breeds extensively in irrigation run-off water and is highly resistant to the organochlorines as well as many of the OP insecticides is particularly serious and with implementation of further irrigation development projects is, in fact, likely to grow worse.

THE PRESENT TRENDS IN MALARIA CONTROL

No additional review beyond what has been presented above will be made of malaria control activities on a country basis. There are, however, general trends in malaria control which can be illustrated.

The 38th World Health Assembly in 1985 adopted a resolution which recommended that malaria control be developed as an integral part of the primary health care system of the endemic countries; it also urged the member states of the WHO to undertake an immediate review and appraisal of their malaria control situation and their existing control strategies in terms of their effectiveness, efficiency and the prospects of achieving and maintaining the objectives of the programmes. It is clear that in the future malaria control programmes and the measures which they use will have to be better adapted to the varied epidemiological situations than has been the case in the past and there is a now much more realization than previously of how greatly the epidemiology of malaria may differ from one area to another. It is also clear that much research remains to be carried out on the local epidemiology of malaria as a basis for the development of more effective control programmes.

While a number of countries have undertaken such reviews, some find their resources too limited to easily depart from the type of control structures that they have long established and which are accepted within the country while still other countries encounter difficulties in the planning of new approaches. Some countries have simply chosen not to change their existing programmes or attempt to plan any new approaches at all.

From the assessment carried out above, it is evident that while there has been some progress in countries with resolute programme, overall the control of malaria has not very greatly improved in the last 4 or 5 years; furthermore, due to financial, political and technical problems, a deterioration has occurred in certain programmes and epidemiological situations.

There are many different reasons for the reduction in effectiveness in control and for the increase in transmission and they may vary greatly from one country to another and from one geographical area to another. The varied causes have been reviewed by the 18th WHO Expert Committee on Malaria in 1985 (WHO, 1986a) and the following section will be based essentially upon that review.

RESPONSES TO CHANGING EPIDEMIOLOGICAL PATTERNS

In most areas outside tropical Africa the present malaria situation is characterized by a marked focal distribution that is often quite different from that which existed before the beginning of control activities (Najera, 1986). Changes in agricultural practices, the migration of rural populations seeking better employment opportunities, increased irrigation as a result of water development projects, the creation of new settlements, usually unplanned, around dams, mining or other major construction areas and the greatly increased urbanization that characterizes most of the developing countries have all resulted in important changes in the epidemiology of malaria. Many programmes have been unable to assess the changes or adapt to them quickly enough to avert the increase in malaria transmission that has often been the consequence of such changes. In some areas, urbanization has reduced malaria transmission in areas of dense urban settlement as there are few larval habitats

suitable for *Anopheles* breeding but often the great increase in peri-urban human populations in areas still close to intense vector mosquito breeding has resulted in increased transmission.

TECHNICAL PROBLEMS FACING MALARIA CONTROL

A number of major technical problems are faced by many malaria control programmes and the inability to adequately deal with them has been among the reasons for the failure of not a few of these programmes. Examples of these technical problems are:

VECTOR RESISTANCE TO INSECTICIDES

Vector resistance to insecticides was one of the earliest of the technical problems to have emerged and remains a major and still growing problem. Vector resistance continues to appear in geographical areas in which it was not previously reported and is emerging in additional species and to additional chemical groups of insecticides. The number of effective alternative compounds that can be considered as replacements for those to which resistance has developed is limited and the prospects for a continued flow of new candidate compounds for field testing from the chemical industry are not very good. The greatly increased costs of pesticide development and the greatly increased toxicological, environmental and ecological strictures on pesticides are among the factors which are reducing the rate of development of new insecticides both for agriculture and for public health use. The generally greater cost of the newer compounds which are now available as compared with that of older insecticides such as DDT, HCH and malathion makes it difficult for programmes already faced with financial stringencies to purchase the newer and more expensive insecticides in the quantities they require.

Multiple resistance to more than one chemical group of insecticides has now developed in at least five important vectors of malaria including *An. albimanus*, *An. culicifacies*, *An. pseudopunctipennis*, *An. sacharovi* and *An. stephensi*. These species or species complexes, show resistance to the organochlorines, OP compounds, carbamates and pyrethroids in one or more geographical areas (WHO, 1986b). Cases of multiple resistance are often associated with the intensive use of many different insecticides in agriculture particularly when they are applied by aircraft to control pests of such cash crops as cotton and rice; run-off irrigation water from the treated fields reaches the breeding sites of anophelines where it may select for insecticide resistance even at times to insecticides or insecticide groups that have not yet been used in public health programmes.

By 1986 a total of 50 different species of anophelines had been recorded as resistant to one or more pesticides in some part of their range. Of these, at least 11 are important species of vectors. Taking into account that some of these are now known to a complex of several different species, the number is actually as high as 57.

Malaria vectors in many areas exhibit an evasive behaviour towards insecticides either by entering sprayed dwellings in smaller numbers or exiting such buildings before acquiring a lethal exposure to the insecticide. In still other areas the exophilic and exophagic habits of some major vector species virtually precludes their control through the application of indoor residual sprays.

PARASITE RESISTANCE TO ANTIMALARIA DRUGS

The development of *P. falciparum* resistance to most of the currently available antimalaria drugs has emerged as the main technical problem in malaria control and has been reported from more than 50 countries. Resistance to chloroquine has spread throughout large areas of South America and Southeast Asia and is now spreading rapidly in Africa after its first appearance in East Africa and more recent appearance in West Africa. In both South America and Southeast Asia the intensity of resistance is also increasing.

Resistance to the sulfadoxine/pyrimethamine drug combination is also spreading and a few instances of resistance to mefloquine have even been noted. The presence of resistant parasites in an area necessitates the procurement of alternative drugs whose cost is greater ranging as high as 33 times greater for sulfadoxine/pyrimethamine as compared to chloroquine curative treatment. Some of the alternative drugs are more likely to produce side effects and their treatment regimens are often longer thus reducing patient compliance.

One of the important reasons for the selection and appearance of resistance in malaria parasites is, in probability, the pressure from the widespread use of antimalarial drugs and their use should be reduced, where practical, to a minimum. In reality, however, antimalarial drugs are almost everywhere commercially available; thus, even if they are not distributed as part of mass drug administration programmes, they will be bought and used, by populations in endemic areas. Frequently they will be used at subcurative doses, as a result of inadequate guidance to their correct use, and this will further increase the selection for resistance.

The wide spread development of drug resistant strains has necessitated the development of a drug response monitoring programme in endemic and non-endemic countries; the processing of the data from these programmes is done by the WHO but the monitoring is the responsibility of countries. The necessity for drug monitoring has considerably increased the cost and complexities of the use of antimalarial drugs in malaria control.

ADMINISTRATIVE AND FINANCIAL PROBLEMS OF MALARIA CONTROL

THE INCREASED COSTS OF MALARIA CONTROL

Increased cost is a frequent consequence of the necessity of replacing insecticides and drugs to which resistance has developed with alternative compounds to which are still effective. For most countries, the cost of malaria control programmes has risen greatly due to increasing costs of transport, fuel and equipment; the higher costs of newer insecticides and drugs, add to a financial burden whose support is already difficult for many countries. The shortage of funds for the purchase and distribution of antimalarial drugs by national health services, eventually results in the populations in endemic areas procuring them through commercial channels and, as has been noted above, such unsupervised use frequently leads to an increase in selection pressure.

Moreover, malaria control programmes in many countries must share the already inadequate funds available to the health services with many other priority programmes in health and development. The generally poor level of development of health services in many of the third world countries, which, unfortunately, are those countries most in need of adequate health service, makes the implementation of new or expanded disease control programmes

unduly expensive; in an increasing number of countries, malaria control activities in the field are being incorporated into the general health services. These health services, however, may find difficulties in sustaining the costs of the malaria control activities which they have taken over especially the cost of insecticides, drugs and other logistic support and often will seek donor funds to procure these items. Where donors choose to support the malaria control activities of the health services, they will often have to consider providing the costs of transport, fuel, insecticides and spraying equipment as well as equipment and supplies for laboratories, clinics and hospitals. Not infrequently, even salaries for additional local personnel engaged in surveillance activities may be beyond the resources of the national health services. When support is required to this extent, the question must be asked as to whether the programme is sustainable at all by the disease endemic country. Donor assistance should, therefore, certainly include help in the planning of the financing of such services as part of long term control programmes.

NEW APPROACHES TO CONTROL STRATEGIES

In 1978, the World Health Assembly endorsed a malaria control strategy based on the principles of primary health care (PHC) and on the recognition of variations in epidemiological situations in different parts of the world and variations in the state of development of health infrastructures in different developing countries. However, even where countries have attempted to integrate malaria control strategies into their PHC programmes while still retaining central specialized services available to the periphery, there have been problems of interpretation, delays in implementation and, in many areas, a reluctance to change long-standing and familiar malaria control practices. Consequently most countries are experiencing difficulties in modifying their established services. Despite these problems, the persistence of the malaria and the cost of traditional control measures are forcing countries to consider the redeployment of their limited resources. Najera (1986) has emphasized that a fully developed health infrastructure is not necessarily a prerequisite to undertaking antimalaria activities. Whatever kind of structural elements exist should collaborate in developing a health infrastructure for primary health care and this infrastructure should view malaria control as an essential component in endemic areas and should distribute appropriate responsibilities to general and specialized health services.

THE EPIDEMIOLOGICAL APPROACH TO MALARIA CONTROL

There are extensive local variations in the intensity of the malaria problem and in the response to control interventions. A better epidemiological understanding of the ecological and social processes interacting with the basic elements of the transmission chain, i.e. parasite, vector, and infected and susceptible host, may enable more selective and effective control measures to be applied at lesser cost.

While measures such as insecticides, and mass drug administration can drastically modify, and reduce malaria prevalence and even eliminate transmission, eventual reduction or cessation of these measures can lead to the restoration of the original level of endemicity. By contrast, less spectacular but more permanent effects on malaria transmission can be obtained by elimination of mosquito breeding places, improvements in house construction,

and, as a consequence of social and economic development, by changing the way of life to ensure that there is an appropriate demand and use of health services.

THE STRATIFICATION OF THE MALARIA PROBLEM

The stratification of malaria problems may be undertaken in a number of different ways; different epidemiological zones can be characterized in terms of their biological and ecological features including those of the vector, the incidence of disease and the social and economic characteristics of the human population. These characterizations can be used to develop a stratification of the control activities to ensure that each area under control has applied to it the approach most appropriate for it and that the resources for control measures are placed in areas that have the greatest need for them coupled with the greatest possibility of utilizing them to achieve effective control.

THE SELECTION OF APPROPRIATE TECHNOLOGIES

The main aim of the epidemiological approach is to identify the appropriate technologies for malaria control as part of PHC. This concept implies that the method selected is scientifically sound for the solution of the given problem in the given place and is adaptable to the society in which it will be used in terms of acceptability and social affordability.

It has been emphasized that antimalaria measures differ considerably in the degree of perfection and coverage required during their application so as to obtain an effect. As an example, coverage by indoor residual insecticide applications must be extensive if they are to have any effect on the protection of the community. By contrast individual measures of protection including the use of bednets with or without insecticide impregnation, screening of dwellings and the seeking of prompt treatment of fevers, reduce the risk to the individual and eventually have an additive effect for the protection of the community.

Where malaria is a serious public health problem, the training of community workers in the diagnosis and treatment of malaria and ensuring their continued support by the health service through the provision of supplies and the referral of problem cases and situations, should also lead to the development of primary health care programmes. By contrast vertical programmes of spraying and mass chemotherapy do not normally engage in development processes. However, antimalarial activities, where possible, should be incorporated into the daily life of the community and the individual to meet a special epidemiological situation. It should be realized that unless control activities are seen to be effective by the community, they may hinder not only their own continuation but the establishment of primary health care.

THE MALARIA CONTROL STRATEGY AND ITS APPLICATION AT THE COUNTRY LEVEL

There are two basically different approaches to malaria control:

- The management of malaria without drastically changing the epidemiological equilibrium including the provision of facilities for diagnosis and treatment and development of epidemiological information systems allowing for focal management

or

- active intervention to control or even interrupt malaria transmission in large areas-

this approach is aimed at developing an improved malaria epidemiological situation that can be sustained.

The choice among the two approaches should be based on the feasibility of maintaining the desired results. As an example, there may be geographical areas in which it is possible to eliminate malaria in a relatively short time. Yet if the same area is likely to be later subject to a recrudescence or a resurgence of malaria and the local health services are unable to maintain a malaria-free state with the resources available to them, little will have been gained by what will have been only a temporary relief from transmission; in fact it can be considered that efforts that achieved only transient results were, in reality, wasteful of resources if populations who, for a time, were protected from the disease were to be later subjected to it anyway.

It must be realized that it will be seldom possible for a country as to adopt a single effective approach to malaria control for all its regions. Where malaria is widespread within a country, planning should be carried out by district level personnel to ensure that it is appropriate to the district level. Generally speaking experience has shown that what is needed to improve malaria control even more than the development of new and improved technologies, is a better understanding of where and how to apply the general and specific knowledge that is already available. The 18th Expert Committee report has recommended in conclusion that:

Countries should review their antimalaria activities in light of their state of development and specific state of development of their PHC structure, taking into account concurrent activities for health promotion and the control of other diseases.

Malaria control should be based on an epidemiological approach which recognizes local variability in the distribution and nature of the problems thus encouraging the selection of suitable control and evaluation measures for different ecological areas.

Planning for antimalaria activities should take into account

- a) ensuring that the best available information and experience is available as a basis for planning and if necessary, acquire additional knowledge;
- b) the relevance of the objectives in terms of their feasibility and their appropriateness with which the basic objective, especially the prevention of mortality is to be attained;
- c) whether the existing infrastructure can ensure total coverage of the population with timely diagnosis, treatment and where necessary, prophylaxis and has facilities for referral for treatment of severe cases and the necessary epidemiological services for detection of critical areas;
- d) the necessity for developing a core group of expertise if this does not already exist and the related training which will be required for the personnel of such groups;
- e) the use of appropriate evaluation and surveillance systems for the monitoring of the progress of malaria control;
- f) the development of methods of integrated vector control especially where they can be carried out and sustained by the community.

THE PROSPECT FOR FUTURE MALARIA CONTROL

There is unlikely to be a simple solution or a panacea for malaria control or for the

control of most of the vector-borne or other communicable diseases for that matter. Even the development of an effective vaccine for malaria would require a national economy capable of acquiring it, an infrastructure capable of ensuring its application, epidemiological services capable of monitoring and evaluating its efficacy at a community level and a health services structure capable of intervening as required in the most appropriate and effective manner. If the principals and steps which have been described and outlined above are followed, the long-term prospects for malaria control are good. However, it must be emphasized again that the objectives and planning for control should be realistic and obtainable. While there is a great need for additional research on drugs, vaccines, and insecticides, and many of the problems of malaria epidemiology and control may eventually be solved by such research, nevertheless a great deal can be achieved by the effective application of principles and methods already known and already available.

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OBSERVATIONS ON THE VALIDITY OF THE OVARIAN ACCESSORY GLANDS OF SEVEN ECUADORIAN SAND FLY SPECIES (DIPTERA: PSYCHODIDAE) IN DETERMINING THEIR PARITY

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Abstract: Females of seven sand fly species caught on man in several leishmaniasis-endemic foci in Ecuador were examined to assess the value of the accessory gland secretions as an indicator of parity. It was found that parous females could be distinguished from nullipars by the presence of granular secretions in the accessory glands in *Lutzomyia ayacuchensis*, probable vector of *Leishmania* in the Andean highlands of southern Ecuador. Examination of the female accessory glands was not a reliable method for determining parity in six other sand fly species caught in lowland areas, including *Lu. trapidoi*, *Lu. hartmanni*, and *Lu. gomezi*, three proven vectors of *Leishmania*, since granular secretions were found in both parous and nulliparous females.

INTRODUCTION

Condition of the ovarioles has proved to be a useful characteristic for distinguishing parous from nulliparous females in several groups of Nematocera, including Phlebotominae (Detinova, 1962). This ovarian method has been thought of as impractical for routine work due to the small size of the ovarioles in sand flies. Examination of the accessory glands of the ovaries, which are large enough to examine quickly, is a useful method for distinguishing parous females in several African sand fly species of the genus *Phlebotomus* (Adler and Theodor, 1935; Lewis and Minter, 1960). Usefulness of this method seems to be dependent upon the sand fly species involved when applied to the New World genus *Lutzomyia*. Accessory gland secretions were reported as a reliable sign of parity in three species from northern California (Chanotis and Anderson, 1967), and also in eight species from Brazil (Lewis *et al.*, 1970), but had no value for determining parity for many other *Lutzomyia* species (Johnson and Hertig, 1961; Johnson *et al.*, 1963; Lewis, 1965; Lewis *et al.*, 1970; Ward, 1974).

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The present study was undertaken to determine if the accessory glands could be used to determine parity of several Ecuadorian sand fly species, including some proven vectors of *Leishmania*.

MATERIALS AND METHODS

Sand fly collections were made at five localities, i.e., Challuabamba and Paute, Department of Azuay, both situated at altitudes of 2,300-2,500 m above sea level in the Andean plateau; Echeandia, Department of Bolivar (altitude ca. 500 m a.s.l.), and Puerto Quito, Department of Pichincha (altitude 450 m a.s.l.), both situated in the western foothills of the Andes; and Paján, Department of Manabi, in the Pacific coastal region (altitude ca. 20 m a.s.l.). All of these localities lie in regions where *Leishmania* is endemic (Hashiguchi, 1987). At Challuabamba and Paute, collections were carried out for three and six nights respectively on rocky hillsides covered in grasses, small shrubs and *Agave* plants. Tree cover was confined to sparse groves of young *Eucalyptus* on the lower slopes.

At the other study sites, sand fly collections were made for one or two nights in secondary forest near human habitation except at Paján, where insects were captured in a coffee plantation.

Flies that alighted on human volunteers between 18.30 and 21.00 were captured using a mouth aspirator (Hashiguchi, 1987) and maintained overnight in small plastic vials provided with sufficient moisture. Female sand flies captured were dissected in saline on a glass slide and the accessory glands checked microscopically for the presence of granular secretions. At the same time, ovaries were examined to determine their parous state, using the follicular relic method of Detinova (1962); the shapes of the spermatheca and cibarium were observed for sand fly identification, which was done using keys of Young (1979) in reference of Cáceres and Bianchi (1988).

The guts of all dissected sand flies were examined for the presence of blood meals. A number of sand flies caught at Challuabamba were allowed to take blood from an anesthetized golden hamster, and kept in a plaster-lined 120 ml plastic vial provided with a piece of sugar cane at a temperature of 15-20°C for three, seven or nine days. The accessory glands of these fed flies were then examined to determine whether granular material had been secreted in accordance with the gonotrophic cycle. The methods of collection of adult sand flies were detailed previously by Hashiguchi *et al.* (1985), and for ovarian dissection by Lewis (1965).

RESULTS

A total of seven sand fly species taken in human bait collections in the five localities sampled were examined. The numbers of females of each species with or without accessory gland secretions, in relation to their parous state as demonstrated by the ovarian method, are shown in Table 1. In *Lu. ayacuchensis* Cáceres and Bianchi from Challuabamba and Paute, granular secretions in the accessory glands were seen in all 27 parous females but not in most nulliparous females.

The nine nulliparous flies with granular secretions had developing follicles of stage IIIa, but these were all among those that had fed on varying quantities of blood after being

Table 1 Granule secretions of accessory glands in relation to parity in seven anthropophilic sand fly species examined during July-August, 1988 in Ecuador

<i>Lutzomyia</i> spp.	Blood meal ¹	No. flies examined	Granule secretions			
			present		absent	
			N ²	P ²	N	P
<i>ayacuchensis</i>						
Challuabamba	—	52	0	7	45	0
	+	12	6	2	4	0
Paute	—	41	0	15	26	0
	+	16	3	3	10	0
<i>trapidoi</i>						
Echeandia	—	84	38	16	28	2
	+	6	6	0	0	0
Puerto Quito	—	11	7	1	3	0
	+					
<i>hartmanni</i>						
Echeandia	—	35	13	13	9	0
	+	5	3	1	1	0
Puerto Quito	—	28	14	8	6	0
	+	4	3	0	1	0
<i>carrerae thula</i>						
Echeandia	—	2	1	1	0	0
<i>panamensis</i>						
Puerto Quito	—	12	4	5	3	0
<i>gomezi</i>						
Paján	—	49	33	16	0	0
	+	14	10	4	0	0
<i>shannoni</i>						
Paján	—	7	2	5	0	0
	+	1	1	0	0	0

1. Fed to a varying degree when collected on men

2. N: nulliparous; P: parous

Table 2 Changes in secretions of accessory glands of *Lutzomyia ayacuchensis* from Challuabamba, fully fed on blood from hamster and kept on sugar cane at a temperature of 14-20°C

Group	Day after blood feeding				
	1	3	5	7	9
fed	4/4 ¹	4/4	5/5	5/5	16/18
	(IIIa) ²	(IIIb)	(IV)	(V) ³	(V) ³
unfed	—	—	0/1	—	0/3
	(—)	(—)	(II)	(—)	(II)

1. No. positive for granule secretions/no. examined

2. Follicular stage

3. Eggs were already deposited in 1 of the 5 females dissected on day 7 and in 17 of the 18 females dissected on day 9.

collected. The granules found in these nulliparous females are therefore considered to have been secreted after the blood meal taken on the preceding day.

In contrast, *Lu. trapidoi* (Fairchild and Hertig), *Lu. hartmanni* (Fairchild and Hertig), *Lu. panamensis* (Shannon) and *Lu. carrerai thula* Young collected from Echeandia and Puerto Quito showed discordant relations between granular secretions and parity. Accessory gland secretions were found in more than half of the nulliparous females, as well as in most of the parous females. All the unfed nulliparous females with granular secretions had follicles of stage I or II.

Accessory gland secretions were seen in all females irrespective of parity, in two *Lutzomyia* species caught from Paján. No developing follicles were seen except in blood-fed flies, which had stage IIIa follicles.

The results of dissections of female *Lu. ayacuchensis* kept for several days after blood feeding are shown in Table 2. This sand fly species was gonotrophically concordant. Egg maturation required approximately seven days, and eggs were laid shortly thereafter. The granules in the accessory glands began to be secreted soon after the blood meal, and were accumulated gradually as follicular development proceeded; the accessory glands enlarged and were filled with dark granules within five days of blood feeding. The accessory glands in 16 of 18 flies which had oviposited had remnants of dark granules in varying amounts, while those in the remaining two females had no residues and resembled those of nullipars. One and three unfed females, examined five and nine days after collection respectively, had no granule secretions and exhibited no follicular development beyond stage II.

DISCUSSION

The present study clearly demonstrates that the accessory glands are a reliable organ for distinguishing parous from nulliparous females of *Lu. ayacuchensis* from Challuabamba and Paute. Granular secretions in the glands of this sand fly were apparently produced only after a blood meal was taken. The changes in the quantity of granular secretions during follicular development and after oviposition are the same as those observed in three Californian sand fly species by Chaniotis and Anderson (1967). It should be remembered that not all females with granular secretions can be judged as parous, since some of the blood-fed nulliparous females might have secreted granules shortly after feeding. In future studies it would therefore be better to discard blood-fed females or to also check the ovarian follicles when examination of these females is required. There is also a possibility that some parous females are mistaken for nulliparous when granules are entirely expelled from the accessory glands after oviposition, as demonstrated in two of the 18 wild-caught females that laid eggs in the laboratory during the present study.

At present little is known about the biology of *Lu. ayacuchensis* or its role in the transmission of *Leishmania* in the Andes of southern Ecuador. This species was first recorded from Ecuador during the present study but originally misidentified as its close relative, *Lu. peruensis* (Shannon). Closer examination revealed that the sand fly fauna of the Paute area consisted of two closely related species, i.e., *Lu. ayacuchensis* and *Lu. osornoi* (Ristorcelli and Van Ty), both of which are anthropophilic and may be involved in transmission of *Leishmania* in the area. Our findings will facilitate future studies on bionomics and dynamics of wild populations of *Lu. ayacuchensis*, and may also be applied in the future to *Lu. osornoi*.

By contrast, the accessory gland secretions did not prove to be useful in distinguishing between parous and nulliparous females for *Lu. trapidoi*, *Lu. hartmanni*, *Lu. panamensis* and *Lu. carrerai thula* collected from Echeandia and Puerto Quito. The first two species have been reported as probable vectors of leishmaniasis in Ecuador (Hashiguchi *et al.*, 1985), while *Lu. panamensis* is a proven vector of *Leishmania* in Panama (WHO, 1984). In each of these four species, granular secretions were seen in most parous females, but more than half of the unfed nulliparous females dissected also had granular secretions. It may be that accessory gland secretions are produced a few days after eclosion, irrespective of whether or not a blood meal is taken, as has been observed in *Lu. longipalpis* (Lutz and Neiva) from Brazil (Ward, 1974).

Accessory gland secretions were observed in all dissected females of *Lu. gomezi* (Nitzulescu), another suspected vector of *Leishmania* in Ecuador (Hashiguchi, 1987), and *Lu. shannoni* (Dyar) from Paján. This indicates that the glands are of no value in determining parity. Lewis *et al.* (1970) reported that some females of *Lu. shannoni* in Belize and *Lu. gomezi*, and three other species in Brazil were probably autogenous, based on the high proportion of flies with granular secretions showing discordant ovarian development. No sign of autogeny was seen in females of the two species collected from human bait in Paján. None of the ovarian follicles in unfed nulliparous females had developed beyond stage II, and accessory gland secretions were found in all these females.

It appears that female accessory gland secretions are not associated with autogeny, and they are probably produced shortly after adult emergence. Autogenous strains have been reported in *Lu. shannoni* from Florida (Perkins, 1982) and in *Lu. gomezi* from Panama (Johnson, 1961). It is possible that autogenous strains of these species also exist in Ecuador. A somewhat high proportion of parous females (five of eight dissected, or 62.5%), among those collected suggests that an autogenous population may be present. Further studies would be required to determine whether this is the case.

At present, there are no reliable methods other than the ovarian relic method in determining reproductive parity of vector sand flies in lowland areas endemic for *Leishmania*. Further efforts are needed to find out reliable methods of distinguishing parous from nulliparous females.

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エクアドルにおける人吸血性サシチョウバエ7種の
卵巣付属腺による顆粒分泌と経産歴との関係

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旧大陸のサシチョウバエでは卵巣付属腺の分泌する顆粒の有無が経産、未経産雌の区別に用いられているが、新大陸のサシチョウバエではこの方法の応用は難しいとされている。今回、エクアドルにおいて人囿法で採集した7種のサシチョウバエを解剖し、ovarian relic法による経産歴と付属腺での顆粒の有無との関係を検討した。低地のリーシュマニア症媒介種、*Lutzomyia trapidoi*, *Lu. hartmanni* および *Lu. gomezi* を含む6種では未経産雌、経産雌いずれにも付属腺の分泌顆粒が見られるなど顆粒の有無だけでは経産歴を判定できないという結果を得た。一方、アンデス高地の *Lu. ayacuchensis* では、分泌顆粒は経産雌のみに見られ、経産個体の判別に用いられ得ることが分かった。

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ECOLOGICAL STUDIES ON THE MOSQUITO FISH,
GAMBUSIA AFFINIS FOR ENCEPHALITIS
CONTROL WITH SPECIAL REFERENCE TO
SELECTIVE FEEDING ON MOSQUITO LARVAE
AND COMPETITION WITH THE MEDAKA,
ORYZIAS LATIPES

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Abstract: The mosquito fish (*Gambusia affinis*) that was transferred to Tokushima City, Tokushima Prefecture in 1969 was studied in relation to the control of mosquitoes. A number of the fish were collected at several ponds and ditches where the fish had increased in number and were repeatedly released into swamps, sewers, fallow rice fields, etc. They were established in 7 places in 1970, 36 in 1971, 76 in 1972 and 104 in 1973. In 27 of those places, both the medaka (*Oryzias latipes*) and mosquito larvae co-existed, but after the establishment of *Gambusia* in these places, no mosquito larvae were found. The population density of *Oryzias* became low and that of *Gambusia* increased. The water areas with high densities of *Gambusia* were still or had an inflow of household waste water causing eutrophy. When *Gambusia* and *Oryzias* were found together in a water area, the total number of fish was as large as 400-500 per 1 m². The result from laboratory observations demonstrated that *Gambusia* prey on more mosquito larvae than *Oryzias*. If *Gambusia* were raised together with *Oryzias* in the same water tank, *Gambusia* attacked and eventually exterminated *Oryzias*. The feeding habits of *Gambusia* and *Oryzias* depend on the availability of food in the environment and are highly variable. It was shown that both species are involved in the competition for space and food in connection with their ecological niche. The above results indicated that *Gambusia* is more suitable for the control of mosquito larvae than *Oryzias*. In fact, it was observed that *Gambusia* became more predominant and more expansive in its habitat in the water areas examined.

INTRODUCTION

The mosquito fish, *Gambusia affinis* (Baird and Girard, 1853) is a viviparous poeciliid fish, the original habitat of which is in the southern part of North America. Since the beginning of this century, it has been exported worldwide, primarily for vector control of malaria. There have been many reports on the results of these vector control attempts, on which Gerberich and Laird (1966) published a review. According to Nakamura (1941), who gave details of its transfer to Japan, *Gambusia* in Japan originated from 405 fish sent from Texas to Hawaii by Mr. Albinseal of Stanford University in 1905, then later transferred to Taiwan

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for the control of *Anopheles*. Mr. H. Komatsu, a goldfish seller, imported approximately 200 *Gambusia* by surface shipment from Taiwan and bred them in Shiga and Wakayama Prefectures in 1916. The descendants of the fish were found in the late 1960's in a swampy area around Tokyo. The habitat of this establishment was described by Okada (1957), Sasa (1972) and others.

In recent years, with the increase of resistance to insecticides in various species of mosquitoes and the increasing problem of environmental pollution, including loss of non target organisms through the use of insecticides, a method of mosquito control that did not rely solely on insecticides became highly desirable. Because of this, the use of fresh water fish such as *Gambusia* has been examined.

The present research was conducted in Tokushima City to examine the usefulness of *Gambusia* in controlling the mosquito, principally *Culex tritaeniorhynchus*, vector of Japanese encephalitis.

Tokushima City, with a population of approximately 230,000, is situated in the delta of the Yoshino River in the eastern part of Shikoku Island. In the 1960's, many cases of Japanese encephalitis occurred in this area. With the aim of controlling vector mosquitoes of the disease, according to an agreement between Tokushima City Medical Association and Tokushima City Authority, a research center (Tokushima City Laboratory for Mosquito Control) was set up in April 1969, and control measures using *Gambusia* against mosquitoes were started.

Since November 1968, a total of 1,000 *Gambusia* were sent to Tokushima City from Tokyo in three lots. They were released and bred in ponds and ditches within the city, and in October of the following year, approximately 25,000 fish were transferred to water areas such as swamps, sewers, fallow rice fields, etc., in 57 places within the city. From the next year onwards, they were continuously collected from breeding places and released elsewhere. As a result, in a short period of five years, *Gambusia* which had been transferred to Tokushima City had greatly expanded their habitat in the swampy area around Tokushima City, and proved to be effective for controlling mosquito larvae. The results were reported partly by Sato *et al.* (1972) and Hirose *et al.* (1978).

The present paper deals with the results of ecological observations regarding the settlement and the expansion of distribution of *Gambusia* in swampy areas where *Culex pipiens pallens*, a vector of filariasis, and *Cx. tritaeniorhynchus*, a vector of Japanese encephalitis, were breeding. The potential efficiency of *Gambusia* for mosquito control will also be compared with that of *Oryzias*, through the studies on the competitive relationship between the two species.

MATERIALS AND METHODS

1. *Gambusia affinis* transferred to Tokushima City

All of *Gambusia* transferred to Tokushima City were sent by air freight from Haneda Airport. The first lot, sent in November 1968, consisted of 200 fish raised by the Department of Parasitology, Institute of Medical Science, The University of Tokyo. The second lot, which arrived on April 17, 1969, and the third lot, which arrived on May 7 in the same year, were collected from the drainage ditch alongside the runway at Haneda Airport (400 each time).

Before the transfer of *Gambusia*, a preliminary survey was made in Tokushima City and the neighboring Naruto City, Komatsujima City, Matsushige Town and Aizumi Town. The results showed that *Oryzias* lived in a widespread area, but there was no evidence of the presence of *Gambusia* in these places.

2. Releasing of *Gambusia affinis*

The 200 *Gambusia* arriving in the first lot were kept in water tanks at the Tokushima City Laboratory for Mosquito Control. Of the 400 fish introduced in the second lot, 130 were released in a pond of 100 m² area and 40 cm depth in Tokushima Castle Park, and 30 were released at each of 2 different places in the sewer in Hachiman-Cho, Tokushima City. Of the 400 fish introduced in the third lot, 220 were released in an artificial pond in Tokushima Castle Park with an area approximately 500 m² and depth approximately 60 cm, 50 in fallow rice fields in Hachiman-Cho and 50 in sewers in Hachiman-Cho. The remaining specimens were kept in tanks at the Laboratory for Mosquito Control.

All of the six water areas mentioned above—three in natural or artificial ponds and three in sewers and fallow rice fields—were chosen as the first sites for releasing *Gambusia*, because the conditions of the sites were expected to provide easy multiplication and recovery. *Gambusia* succeeded in settling in all of these water areas.

Between July and October 1969, some fish were captured and the second release was made at 63 sites in sewers and swampy areas, mainly within Tokushima City. Afterwards, the third release of fish was made in 73 places (1970), the fourth release in 132 places (1971), and the fifth release in 74 places (1972). The number of fish released was from 50 to 100 depending on the size of water area.

3. Investigations regarding the distribution, population density, body length and feeding habit of *Gambusia affinis* and *Oryzias latipes*

1) Distribution:

In a large number of water areas in Tokushima City, investigations of the distribution of *Gambusia* and *Oryzias* were conducted. In the present paper only the results of studies in those areas where the investigations were conducted regularly are mentioned.

For most density determinations, fish collection was by a four-arm scoop-net (1 m × 1 m) applied five times at each site, and the total number of fish was counted. In small water areas where this scoop-net did not fit, a dipnet, 25 cm in diameter, was used for sampling five times.

2) Population density:

The population density was investigated in the swamp at Kita-Okinosu. This area is in a brackish water zone, where eularias (*Phragmites communis*) grows, and is a typically productive breeding place for *Gambusia* in Tokushima City. The following two methods were used for density determination;

a. Mark-and-release method. Specimens collected from the water area under investigation were held in a 1:10,000 water solution of neutral-red for 10-20 min for marking, and then released. When marked and unmarked specimens were completely mixed after about 1 hr, they were captured again by scooping, and the number of individuals was estimated using the Lincoln index method. The population density was estimated by this method on April 17, July 27 and September 21, 1972.

b. Scooping method. The 1 m × 1 m four-arm scoop-net was put into the water for 15 min and fish were scooped up and counted. This was repeated at 5 different places in each

water area and an average was calculated, on April 18, July 28 and September 22, 1972.

3) Body length:

All the specimens obtained by the scooping method in the Kita-Okinosu swamp were used for measurements of body length.

4) Feeding habit:

Gambusia and *Oryzias* from Kita-Okinosu and Showa-Cho, two representative water areas in Tokushima City in which *Gambusia* had settled were examined. The specimens collected were immediately submerged in a solution of alcohol (70%) and formalin (10%), and stomach contents were analyzed.

4. Comparative examination of the ability of *Gambusia affinis* and *Oryzias latipes* to prey on mosquito larvae and other fish

1) The number of mosquito larvae preyed on:

One pair of *Gambusia* and one pair of *Oryzias* were observed in a 2,500 ml plastic container, provided with 100 fourth instar larvae of *Culex pipiens pallens* every 24 hr, and the number of larvae ingested by each pair was counted. Together with mosquito larvae, solid synthetic food (MF mouse food, Oriental Yeast Co.) was given to Group B and D, but not to Group A and C. Three days before the experiment, food supplies to both fish species were stopped. The average body length of the *Gambusia* was 2.6 cm for females and 2.4 cm for males, while that of the *Oryzias* was 2.5 cm for females and 2.7 cm for males. The experiment was repeated 5 times.

2) Preference in feeding on mosquito larvae or young guppies (*Poecilia reticulata*):

Ten young *Poecilia* were placed in a 2,500 ml container with or without 0.02 g solid synthetic food and 100 mosquito larvae, and the preference of *Gambusia* and *Oryzias* for *Poecilia* or mosquito larvae was compared. Ten young *Poecilia*, only, were released into container number 6 was observed as a control. *Gambusia* and *Oryzias* were not fed for three days prior to the experiment. The solid synthetic food was provided daily. The mosquito larvae used were *Cx. pipiens pallens* (fourth instar). These were replaced daily. Ten young *Poecilia* were introduced only at the beginning, and observed for the number surviving each day.

The average body length of *Gambusia* used in the experiment was 2.6 cm for females and 2.5 cm for males and that of *Oryzias* was 2.6 cm and 2.7 cm, respectively. The average body length of young *Poecilia* was 0.7 cm.

3) Competition for survival in a small space:

Gambusia and *Oryzias* in ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 were released into 2,500 ml plastic containers. Total number of both species was fixed at 20, and the sex ratio was 1:1. During the experiment they were given 0.2 g solid food daily and the numbers of both fish were observed every day. Three days prior to the experiment, food supplies were stopped. The average length of *Gambusia* was 2.6 cm for females and 2.5 cm for males, and that of *Oryzias* 2.6 cm for females and 2.8 cm for males.

RESULTS

1. Establishment of *Gambusia affinis*

Figure 1 shows the main habitats of *Gambusia affinis* in Tokushima City in 1973. It is apparent that *Gambusia* were found to be distributed in most parts of the city four years after

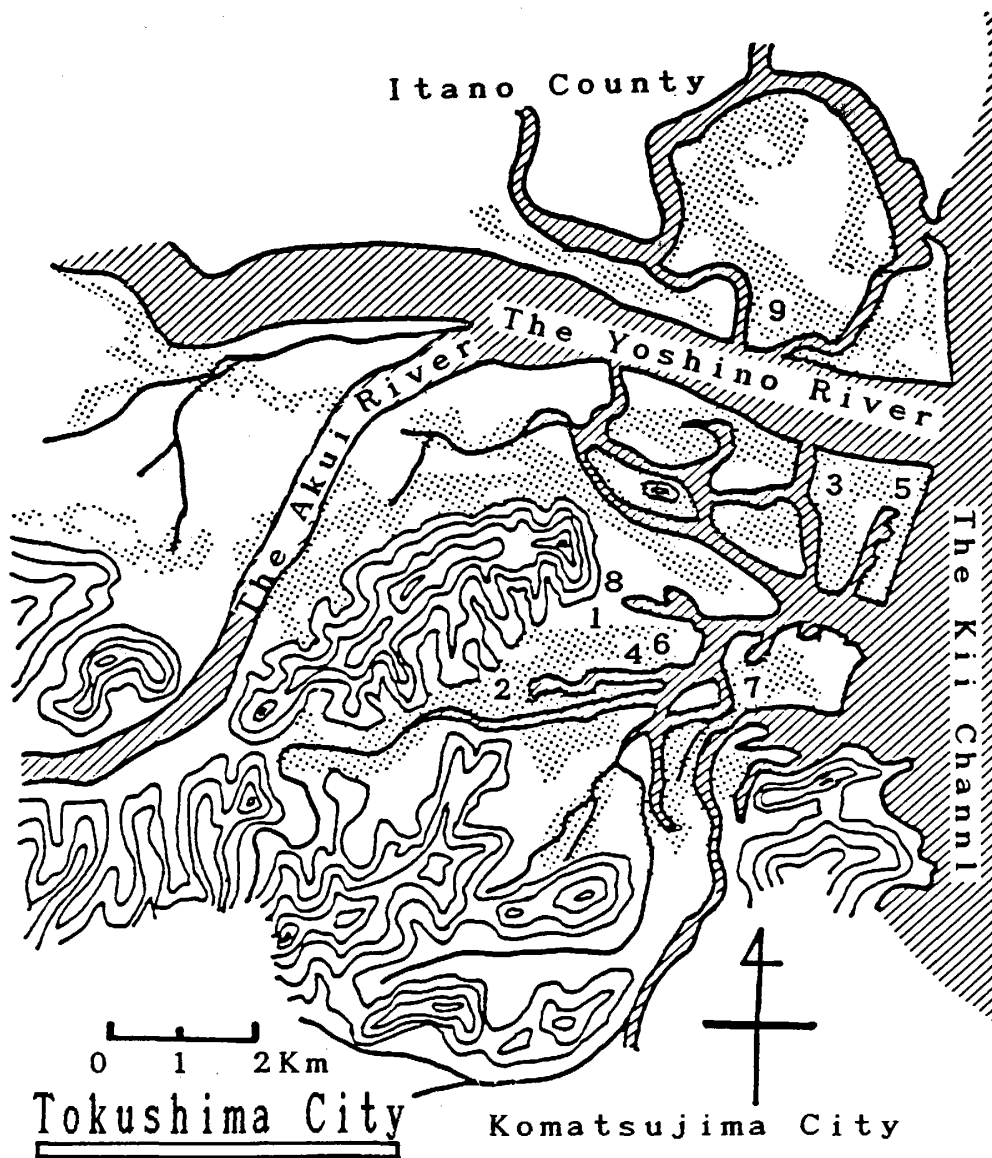


Figure 1 Main habitats of *Gambusia affinis* in Tokushima City. Place numbers are the same as shown in Figure 2 and Table 3.

▨ : River and sea.

▤ : Breeding places of *Gambusia affinis* in Oct., 1973.

1. Pond, Tokushima Castle Park (Settling was confirmed in Aug., 1969).
2. Fallow rice field, Hachiman-Cho (Settling was confirmed in Aug., 1969).
3. Swamp, Kita-Okinosu (Settling was confirmed in Aug., 1970).
4. Swamp, Yamashiro-Cho (Settling was confirmed in Aug., 1971).
5. Agricultural conduit, Kanazawa-Shinden (Settling was confirmed in Aug., 1971).
6. Agricultural conduit, Yamashiro-Cho (Settling was confirmed in Aug., 1971).
7. Swamp, Tsuda-Cho (Settling was confirmed in Oct., 1971).
8. Maot, Tokushima Castle Park (Settling was confirmed in Oct., 1971).
9. Agricultural conduit, Kawauchi-Cho (Settling was confirmed in May, 1973).

Table 1 Establishment of *Gambusia affinis* during five years

Year	1969*	1970*	1971*	1972	1973
No. of places where <i>Gambusia</i> were released	6	63	73	132	74
No. of places where <i>Gambusia</i> were found breeding					
Ponds & moats	3	3	3	3	3
Agricultural conduits & sewers	2	2	16	49	67
Fallow rice fields	1	1	4	7	8
Swamps	0	1	13	20	26
Total	6	7	36	79	104

* : Cited from Sato *et al.* (1972).

Table 2 Influence of the release of *Gambusia affinis* on mosquito larvae and *Oryzias latipes* in 1969-1973

	No. of places					
	<i>Gambusia</i> settled	mosquito larvae		<i>Oryzias</i> larvae		mosquito larvae & <i>Oryzias</i> existed*
		existed*	disappeared**	existed*	disappeared**	
Ponds & moats	3	0	0	3	1	0
Agricultural conduits & sewers	67	47	33	67	44	3
Fallow rice fields	8	8	7	8	5	3
Swamps	26	21	21	26	0	21
Total	104	76	61	104	50	27

* : Before releasing, ** : After releasing.

Data in 1969-1971 were cited from Sato *et al.* (1972).

Table 3 Water quality of the main habitats of *Gambusia affinis* from October 1970 to October 1973

Place	Temp (°C)			pH			Transparency			DO (O ₂ mg/l)			Cl'	Other fishes
	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.		
1. Pond (Tokushima Castle Park)	18.3	22.5	28.8	6.8	6.9	7.2	20.0	25.7	28.0	8.24	8.80	9.35	1,210	<i>Procambarus</i>
2. Fallow rice field (Hachiman-Cho)	18.8	23.8	33.7	6.2	6.7	7.0	7.0	9.8	15.0	2.33	3.45	5.19	—	<i>Procambarus</i>
3. Swamp (Kita-Okinosu)	18.9	24.0	34.0	7.0	7.7	10.8	4.0	9.8	15.5	2.5	4.31	7.14	14,800*	<i>Mugil</i>
4. Swamp (Yamashiro-Cho)	18.3	24.4	28.7	5.6	6.5	7.2	26.0	28.6	30<	3.98	5.91	8.49	15,200	—
5. Agricultural conduit (Kanazawa-Shinden)	20.0	26.3	30.0	6.8	7.6	8.2	12.0	20.2	28.0	5.64	7.9	9.71	14,500*	—
6. Agricultural conduit (Yamashiro-Cho)	18.5	22.1	29.0	6.8	7.8	10.0	5.2	11.7	18.0	3.14	5.98	8.32	14,200	<i>Carassius</i>
7. Swamp (Tsuda-Cho)	18.3	22.3	26.8	6.2	6.7	7.4	8.5	14.1	21.0	1.78	3.36	5.92	1,430	<i>Carassius</i>
8. Maot (Tokushima Castle Park)	20.5	25.0	29.7	5.8	6.5	6.8	20.9	28.0	30<	8.88	9.56	10.90	16,700	—
9. Agricultural conduit (Kawauchi-Cho)	18.7	23.8	30.1	6.2	7.0	7.6	12.5	21.2	30<	2.18	6.08	9.51	1,410*	<i>Carassius</i>

Measurement or observation was made 7-10 times, except for DO 3-7 times and Cl' once.

Place numbers are the same as in Fig. 1., Cl' data with* marked were cited from Sato *et al.* (1972).

the start of the release program. Of the six places where *Gambusia* were first released in 1969, they became established in all the places (Table 1). Young *Gambusia* were observed already in May 1969, and they developed to maturity between July and October of the same year. Some full grown fish were collected and released again in 63 places in the following year, 1970. The results of investigations conducted between May and October 1970 showed that they settled in 7 places, only one more than in the previous year. In the same year, the third release of fish was made at 73 places, and by 1971, *Gambusia* had settled in 36 places. Release were continued in the same way; the fourth in 1971 and the fifth in 1972. As a result, *Gambusia* became established in 79 and 104 places in 1972 and in 1973, respectively. In October 1973 they were found at many places in the delta zone near the central part of Tokushima City. By this time, they were so widely distributed that it was difficult to count the number of places in which they were living.

After the start of *Gambusia* breeding, mosquito larvae disappeared in 61 out of 76 places (80.3%). In 27 out of 61 places, *Oryzias* and mosquito larvae co-existed at first. In all of these places, where *Gambusia* were established, mosquito larvae disappeared (Table 2). Other places where mosquito larvae continued to develop after the establishment of *Gambusia* were usually agricultural conduits and sewers. In the former water areas, *Oryzias*, the motsugo (*Pseudorasbora parva*), the crucian carp (*Carassius carassius buergeri*), etc., were present, so that *Gambusia* were unable to densely populate the area; or, in other places, luxuriant growth of duckweed, etc. protected the mosquito larvae from predation. In the latter, mosquito larvae were often found living amongst drifting material such as rubbish.

Table 3 shows the quality of water regularly examined in the main habitats of *Gambusia*. These water areas share the characteristics of a weak current, an inflow of household water or brackish water, and eutrophy.

2. Changes in distribution of *Gambusia affinis* and *Oryzias latipes*

The changes in distribution of *Oryzias* in 9 main water areas, where *Gambusia* settled (1969-1973), are shown in Figure 2. Places No. 1, and 2, chosen as the first release areas for multiplication, were water areas originally inhabited by *Oryzias*, but by August 1969, *Gambusia* bred sufficiently to allow collection of the fish for the second release.

Gambusia quickly increased in number in most places, but the increase was delayed when the density of *Oryzias* was high (places No. 7 and 9). In place No. 8, *Gambusia* lived for only two seasons, and at the time of concluding this research in October 1973, they could no longer be found there. The reason for this failure was thought to be the unsuitable quality of the water for breeding. As a consequence of the establishment of *Gambusia*, *Oryzias* were reduced in distribution.

3. Population density and body length of *Gambusia affinis* and *Oryzias latipes*

1) Population density:

Table 4 shows the population densities of *Gambusia affinis* and *Oryzias latipes* at a swamp in Kita-Okinosu. The densities differed according to whether the method of measurement was the mark-and-release method or the scooping method. The estimated density of *Gambusia* per 1 m² was 377 by the former method and 76 by the latter in April, 504 and 113 in July, and 488 and 1,018 in September. Likewise, in the case of *Oryzias*, the figure was 2 by the mark-and-release method and 23 by the scooping method in April, 5 and 29 in July, while in September only one fish was counted using the former method. The reason for this discrepancy in densities determined by the two method was not clear, but it is certain that the

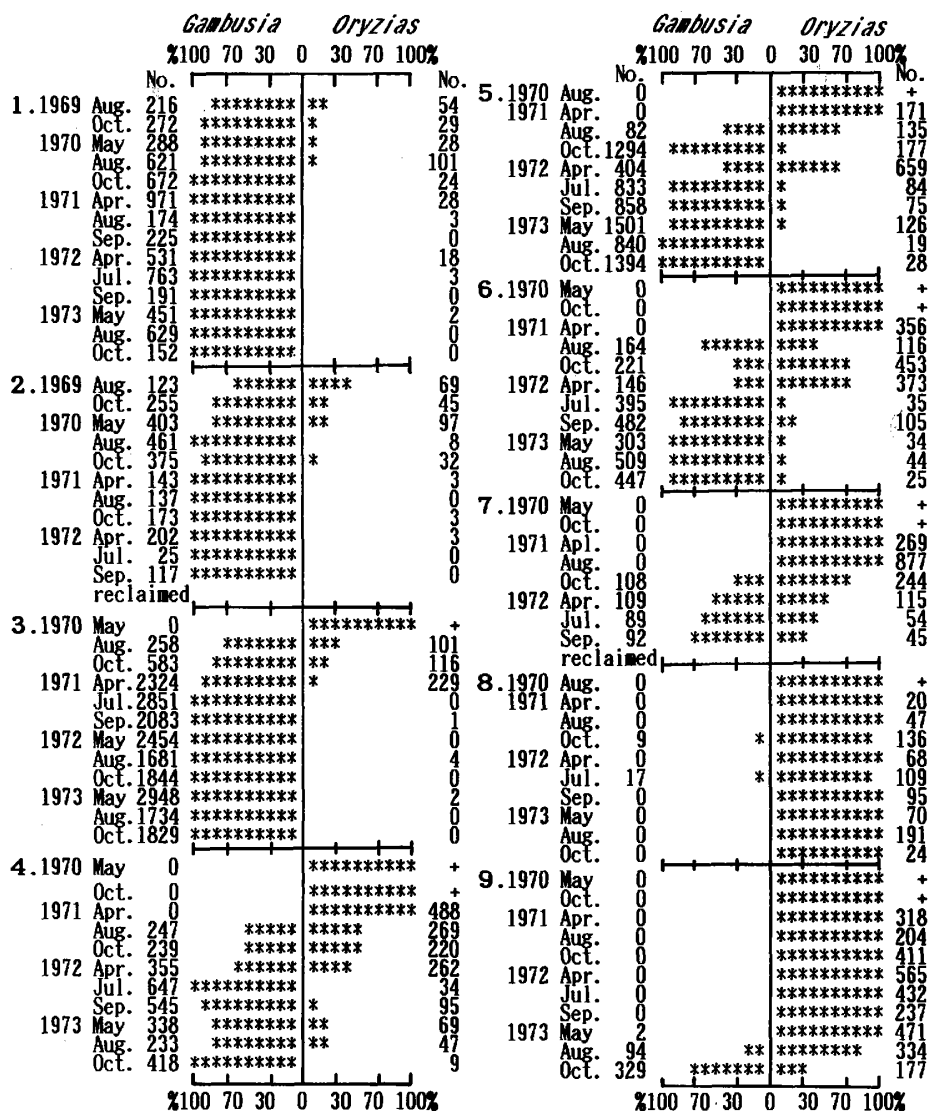


Figure 2 The changes in distribution of *Gambusia affinis* and *Oryzias latipes* in the main water areas where released *Gambusia affinis* settled. Place number are the same as in Figure 1.

One * shows 10%.

No. : Number of fish, four-arm scoop-net or dipnet was used five times at each site.

+ : *Oryzias latipes* were captured but the number was not counted.

1. Pond, Tokushima Castle Park (Releasing was in Apr., 1969).
2. Fallow rice field, Hachiman-Cho (Releasing was in May, 1969).
3. Swamp, Kita-Okinosu (Releasing was in May, 1970).
4. Swamp, Yamashiro-Cho (Releasing was in May, 1970).
5. Agricultural conduit, Kanazawa-Shinden (Releasing was in Aug., 1970).
6. Agricultural conduit, Yamashiro-Cho (Releasing was in May, 1970).
7. Swamp, Tsuda-Cho (Releasing was in May, 1970).
8. Moat, Tokushima Castle Park (Releasing was in Aug., 1969).
9. Agricultural conduit, Kawauchi-Cho (Releasing was in May, 1970).

Table 4 Population densities of *Gambusia affinis* and *Oryzias latipes* estimated by mark-and-release method and scooping method

Method	Mark-and-release						Scooping						
	<i>Gambusia</i>			<i>Oryzias</i>			<i>Gambusia</i>				<i>Oryzias</i>		
Species													
Sex	Female	Male	Total	Female	Male	Total	Female	Male	Fry	Total	Female	Male	Total
April													
No. marked & released	280	121	401	7	4	11	—	—	—	—	—	—	—
No. captured	945	621	1,566	23	15	38	353**	28**	0**	381**	61**	55**	116**
No. recaptured	34	27	61	4	3	7	—	—	—	—	—	—	—
Total No. in swamp*	7,782.4 ±1,310.4	2,783.0 ± 523.8	10,565.4 ±1,834.2	40.3 ±18.3	20.0 ±10.3	60.3 ±28.6	1,976.8 ±678.0	156.8 ±58.8	—	2,133.6 ±736.8	341.6 ±84.0	308.0 ±20.4	649.6 ±104.4
Density per 1 m ² **	277.9 ± 46.8	99.4 ±18.7	377.3 ± 65.5	1.4 ±0.7	0.7 ±0.4	2.1 ±1.1	70.6 ±24.2	5.6 ±2.1	—	76.2 ±26.3	12.2 ±3.0	11.0 ±4.3	23.2 ±7.3
July													
No. marked & released	376	361	737	6	5	11	—	—	—	—	—	—	—
No. captured	1,262	677	1,939	12	13	25	234**	154**	177**	565**	72**	72**	144**
No. recaptured	71	32	103	1	1	2	—	—	—	—	—	—	—
Total No. in swamp*	6,683.3 ± 770.5	7,416.9 ±1,240.6	14,100.2 ±2,011.1	72.0 ±68.9	65.0 ±62.4	137.0 ±131.3	1,310.4 ±450.8	862.4 ±375.2	991.2 ±453.6	3,164.0 ±1,279.6	403.2 ±162.4	403.2 ±165.2	806.4 ±327.6
Density per 1 m ² **	238.7 ± 27.5	264.9 ± 44.3	503.6 ± 71.8	2.6 ±2.5	2.3 ±2.2	4.9 ±4.7	46.8 ±16.1	30.8 ±13.4	35.4 ±16.2	113.0 ±45.7	14.4 ±5.8	14.4 ±5.9	28.8 ±11.7
September													
No. marked & released	341	197	538	1	0	1	—	—	—	—	—	—	—
No. captured	647	319	966	1	0	1	3,270**	1,627**	195**	5,092**	0**	0**	0**
No. recaptured	19	24	43	0	0	0	—	—	—	—	—	—	—
Total No. in swamp*	11,048.4 ±2,373.5	2,618.5 ± 514.0	13,666.9 ±2,887.5	—	—	—	18,312.0 ±15,210.0	9,111.2 ±4,860.8	1,092.0 ±551.2	28,515.2 ±20,622.0	—	—	—
Density per 1 m ² **	394.6 ± 85	93.5 ±18.4	488.1 ±103.4	—	—	—	654.0 ±543.2	325.4 ±173.6	39.0 ±19.7	1,018.4 ±736.5	—	—	—

*: ± variance

** :total No. scooped 5 times

Location: Swamp at Kita-Okinosu (water depth 0.30~0.7 m, width 2.0 m, length 14 m)

Date of investigation: Marking method (Apr. 17, Jul. 27, Sep. 20 in 1972)

Scooping method (Apr. 18, Jul. 28, Sep. 21 in 1972)

In the mark-and-release method, the total number, \hat{N} , and its variance, $V(\hat{N})$, were estimated by $\hat{N} = CR/r$ and $V(\hat{N}) = R^2C(C-r)/r^3$, where R is the number marked and released, C the number captured, and r the number recaptured. If r/R was less than 0.1, following equation was used. $\hat{N} = (C+1)R/(r+1)$; $V(\hat{N}) = R^2(C+1)(C-r)/(r+1)^2(r+2)$

densities were quite high at peak season.

2) Body length:

In spring, body length of *Gambusia* females were larger than those of males, indicating that they were fully matured. As shown in Figure 3, *Gambusia* males in April were small, but the majority were considered to have survived the winter, because immature *Gambusia* were not found in this season. By July, presumably most of the old fish had died, and a number of immature fish had emerged, because the average size was small and a similar distribution in body size was seen in both sexes. In September, the body size became larger than in July, and immature *Gambusia* were also observed. The results of observations mentioned above seem to indicate that the generation turnover occurs mostly in one year in the field, though the

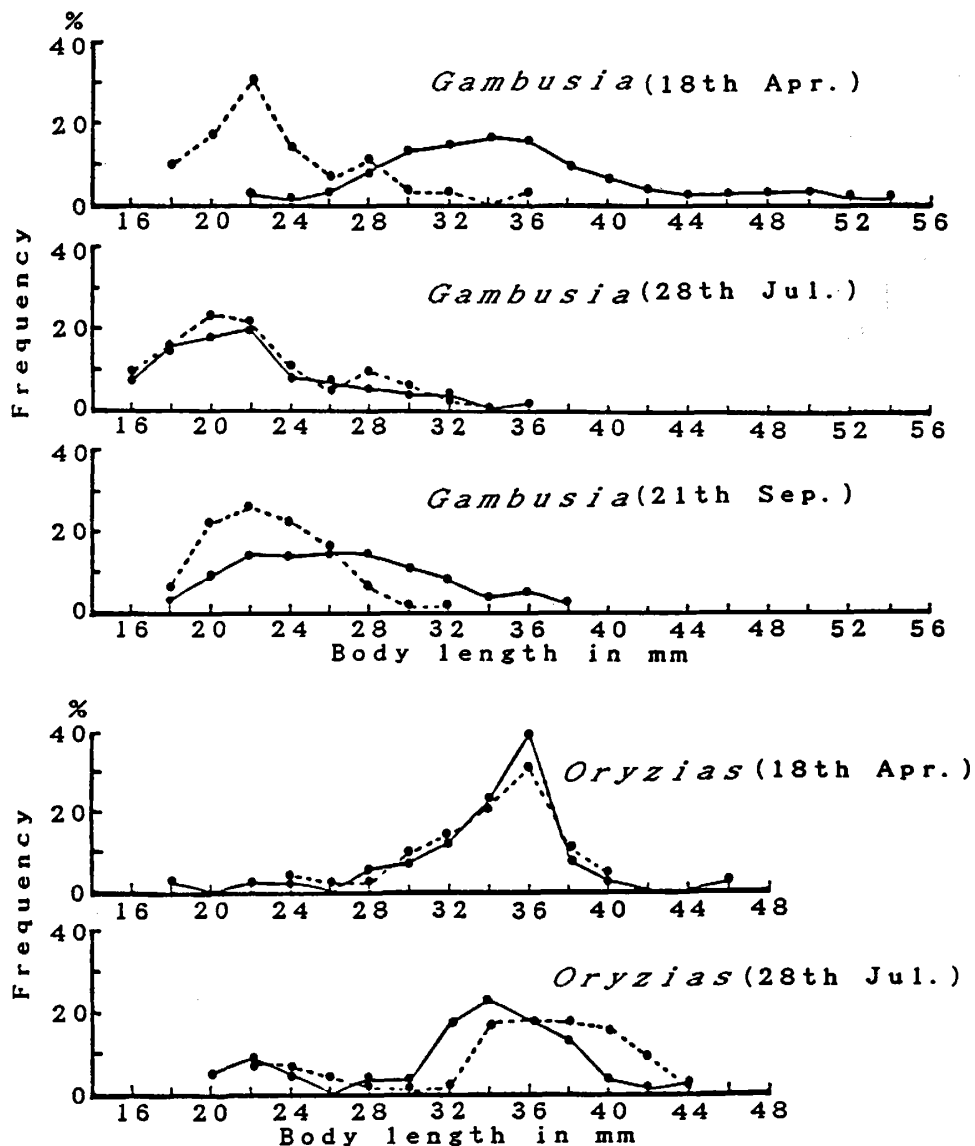


Figure 3 Distribution of body length in *Gambusia affinis* and *Oryzias latipes*. Solid and dotted lines indicate females and males, respectively.

average life span is 2-3 years in the laboratory.

In the case of *Oryzias*, immature fish were absent and the body length of males and females were similar to each other in April and July. In September, no specimens could be caught. From these observations, the life cycle could not be clearly understood from the frequency distribution of body length, though it may be similar to that of *Gambusia*.

4. Feeding habits

Table 5 shows the results of the stomach contents that were studied from the specimens of *Gambusia* and *Oryzias* collected from a swamp in Kita-Okinosu (on August 24 in 1972) and a fallow rice field in Showa-Cho (on July 21 in 1973). The characteristic result showed that *Gambusia* fed on Asplanchnidae, Euplotidae, adult Chironomidae and wing of Coleoptera, whereas *Oryzias* fed on pollen with anther and vegetable fibers in the swamp. In the fallow

Table 5 Stomach contents of *Gambusia affinis* and *Oryzias latipes* in the main habitats

Contents		(1) Kita-Okinosu		(2) Showa-Cho	
		<i>Gambusia</i>	<i>Oryzias</i>	<i>Gambusia</i>	<i>Oryzias</i>
Cyanophyceae	<i>Oscillatoria</i>	r	r	—	r
Diatomeae	<i>Navicula</i>	++	++	r	+
"	<i>Nitzschia</i>	+	+	—	—
"	Others	—	—	r	r
Chlorophyceae	<i>Scenedesmus</i>	r	r	r	+
"	Others	—	—	r	+
Protozoa	<i>Eudorina</i>	r	—	—	r
Trochelminthes	<i>Trichocerca</i>	—	—	r	r
Asplanchnidae	<i>Asplanchna</i>	r	—	—	—
Daphniidae	<i>Moina</i>	—	—	r	r
Centropagidae	<i>Sinocalanus</i>	++	++	—	—
"	Others	r	—	+	+
Euplotidae	<i>Stylonychia</i>	r	—	—	—
Culicidae	<i>Culex pipiens</i>	r	—	+	r
"	(adult) Others	—	—	r	—
Chironomidae (larvae)		r	—	r	r
"	(adult)	r	—	—	—
Formicidae		—	—	r	—
Odonata (leg of larva)		—	—	r	—
Coleoptera (wing)		r	—	—	—
Lepidoptera (scales)		—	—	r	—
scale of fish		r	—	—	—
larvae of shellfish		—	—	r	—
pollen with anther		—	r	—	—
vegetable fibers		—	+	—	r

(1) : Swamp, collected Aug. 24, 1972.

(2) : Fallow rice field, collected Jul. 21, 1973.

In each place, 5 fish were dissected respectively.

r: 1~3., +: 4~10., ++: 11<.

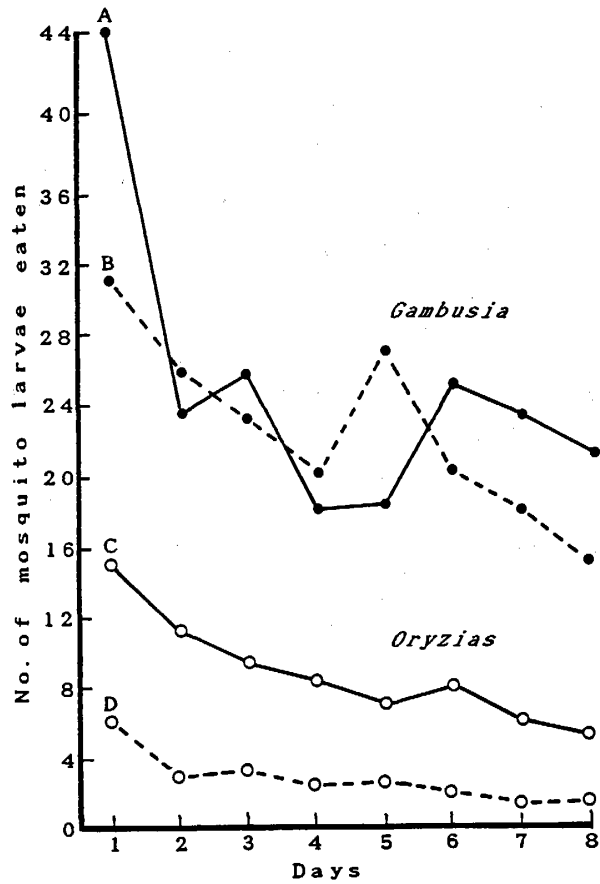


Figure 4 Changes in mean number of mosquito larvae eaten by one pair of *Gambusia affinis* and *Oryzias latipes*.

A, C (Solid line): Solid synthetic food was not given.
 B, D (Dotted line): Solid synthetic food was given.

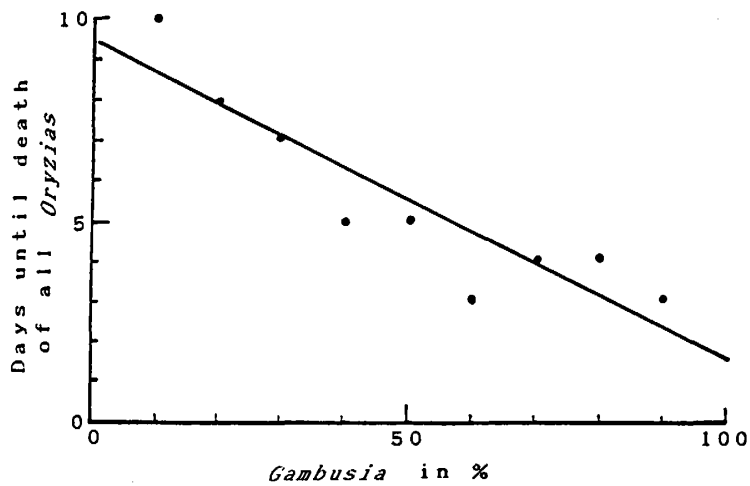


Figure 5 Relationship between the time in days until all *Oryzias latipes* were killed and the ratio of *Gambusia affinis* to all fish in %, when the two fish species were kept together in a 2,500 ml plastic container.

Table 6 Predation of young *Poecilia* and mosquito larvae by *Gambusia* and *Oryzias*

Container	1		2		3	4	5		6 (control)
Fish	1 pair of <i>Gambusia</i>		1 ♀ of <i>Gambusia</i>		1 ♀ of <i>Gambusia</i>	1 pair of <i>Oryzias</i>	3 pairs of <i>Oryzias</i>		10 <i>Poecilia</i>
Conditions	0.02 g solid food 100 mosquito larvae 10 <i>Poecilia</i>		— 100 mosquito larvae 10 <i>Poecilia</i>		— — 10 <i>Poecilia</i>	— — 10 <i>Poecilia</i>	0.02 g solid food 100 mosquito larvae 10 <i>Poecilia</i>		0.02 g solid food — —
Days	<i>Poecilia</i>	mosquitoes	<i>Poecilia</i>	mosquitoes	<i>Poecilia</i>	<i>Poecilia</i>	<i>Poecilia</i>	mosquitoes	<i>Poecilia</i>
1	9/10	82/100	0/10	100/100	10/10	0/10	0/10	60/100	0/10
2	1/1	100/100	3/10	74/100	—	0/10	0/10	32/100	0/10
3	—	—	7/7	27/100	—	0/10	0/10	14/100	0/10
4	—	—	—	—	—	4/10	1/10	21/100	0/10
5	—	—	—	—	—	2/6	0/9	13/100	0/10
6	—	—	—	—	—	1/4	0/9	supply	0/10
7	—	—	—	—	—	3/3	0/9	stopped	1/10
8	—	—	—	—	—	—	1/9	—	0/9
9	—	—	—	—	—	—	0/8	—	0/9
10	—	—	—	—	—	—	4/8	—	0/9
11	—	—	—	—	—	—	2/4	—	0/9
12	—	—	—	—	—	—	2/2	—	0/9

Numbers of eaten/Numbers of supplied

rice field, *Gambusia* fed on adult Culicidae, Formicidae scales of Lepidoptera and larvae of shellfish, and *Oryzias* fed on larvae of shellfish and vegetable fibers. Although both species are apparently euryphagous, *Gambusia* seemed to prefer more animal content in its diet.

5. *Observations of Gambusia affinis and Oryzias latipes preying on mosquito larvae and other fish*

1) Preying on mosquito larvae:

As shown in Figure 4, *Gambusia* ate more mosquito larvae than *Oryzias*. For *Oryzias*, the number of mosquito larvae eaten was smaller when solid food was provided, but for *Gambusia* there was no significant change in diet. Both species of fish ate many mosquito larvae on the first and second days. This is because food supplies were stopped for three days before the experiment. It was concluded that *Gambusia* normally eats many more mosquito larvae than *Oryzias*, whether or not other food is supplemented.

2) Predation on young *Poecilia* and mosquito larvae:

As shown in Table 6, *Gambusia* in container No. 1 ate 82 mosquito larvae and 9 young *Poecilia* on the first day. On the second day, they ate all of the mosquito larvae and one remaining *Poecilia*. *Gambusia* in container No. 2 did not eat *Poecilia* at all on the first day, but consumed all the mosquito larvae. The fish in container No. 3 ate all of the young *Poecilia* on the first day.

On the other hand, *Oryzias* in container No. 5 ate 60 mosquito larvae on the first day, but did not consume much after that. However, on the eighth day, 2 days after we stopped giving them mosquito larvae and solid food, they began to attack young *Poecilia*, and by the twelfth day consumed all of them. Fish in container No. 4 ate 4 young *Poecilia* on the fourth day, 2 on the fifth day, 1 on the sixth day, and the remaining 3 on the seventh day.

The results show that *Gambusia* prey on young *Poecilia* even when solid food and mosquito larvae are present, but *Oryzias* do not prey on young *Poecilia* if they have other food. Thus, *Gambusia* feed on mosquito larvae and young *Poecilia* more than *Oryzias*.

3) Competition for survival between two fishes in a small space:

As shown in Figure 5, *Oryzias* were attacked and eaten by *Gambusia*, and all of the former died by the tenth day, even when the ratio of *Gambusia* to *Oryzias* was 1:9. The relation between the time, in days, until all *Oryzias* were killed (Y) and the ratio of *Gambusia* to all fish in % (X) was linear and expressed by the regression line $Y = -0.08X + 9.4444$ (correlation coefficient $r = -0.9115$). Thus it was shown that in the situation of this experiment, *Gambusia* is clearly more aggressive than *Oryzias*.

DISCUSSION

Gambusia affinis transferred to Tokushima City established and bred within a year, and their distribution spread naturally over wide areas within 5 years. However, when *Gambusia* were released into environments in which other fish such as *Oryzias*, *Carassius* and *Pseudorasbora* were abundant, *Gambusia* sometimes could not easily establish nor breed. This is thought to be due to the small number of *Gambusia* used for releases. However, *Gambusia* finally became most abundant in every kind of water areas.

According to the author's observation, when *Gambusia affinis* and *Poecilia reticulata* were released together into the water area where *Oryzias latipes* were already present and household waste water flowed in, *Poecilia* separately lived in places nearest to household waste

water escape, *Oryzias* lived nearest to swamps and irrigated fields and *Gambusia* lived in the middle. Thus, the *Gambusia* is better able to adapt to still water areas in eutrophy. In addition, *Gambusia* is more aggressive than *Oryzias*. This is the reason why they were able to settle the delta zone of Tokushima City within a short period. A water area, No. 8 (moat), in which they did not establish, had a rather strong current, and thus was an unsuitable environment for *Gambusia*.

Gambusia raised in the laboratory usually live for 2 or 3 years, but the generation is thought to change yearly in the environment in Tokushima City. In July, large *Gambusia* disappeared, and small fish, including immature ones, increased. In September, the number of young and large *Gambusia* increased and the body length became variable, but a few specimens were as big as those observed in April. This seasonal change of population composition seems to indicate that the life span in the field is about one year.

In the area where *Gambusia* and *Oryzias* were mixed, the density of *Oryzias* was high in the spring, whereas in summer and autumn the number of *Gambusia* was large. This transition in the population density occurs because of different breeding seasons, *Oryzias* breeding earlier. Sato *et al.* (1972) reported that *Gambusia* tend to maintain a higher population density than *Oryzias*, and that the total population of the two species was about 300-500 per 1 m², whether they are separated or mixed in distribution. In the present study, the density was similar, about 400-500. When the density of *Oryzias* is already at this level, it is more likely to take a long time for the introduced *Gambusia* to establish and become abundant. Therefore, in such a situation, a large number of *Gambusia* would need to be released to obtain successful establishment.

It was not unusual for mosquito larvae and *Oryzias* to co-exist. However, mosquito larvae could not live with *Gambusia* in the same water area, unless *Gambusia* access to larvae was impaired by rubbish, waterweeds, etc. (Sato *et al.*, 1972). In the laboratory experiment, *Gambusia* consumed a lot of mosquito larvae, but *Oryzias* did not actively feed on them nor show aggressive activity to other fish such as *Poecilia reticulata*, if other nourishment, e.g. solid food, was available. *Gambusia*, on the other hand, attacked and exterminated not only *Poecilia* fry but also mature *Oryzias* when kept in a small space in the laboratory, whether or not other food was available. The same phenomenon has often been observed in the field. This characteristic is significant in terms of the ability to establish in the natural habitat of *Oryzias* in Tokushima City.

There are many reports on the feeding habits of viviparous poecillid fishes (Hess and Tarzwell, 1942; Harrington and Harrington, 1961; Yamagishi, 1966; Yamagishi *et al.*, 1966, 1967; Hubbs, 1971; Sholdt *et al.*, 1972; Sawara, 1974), and generally *Gambusia* is said to be euryphagous. The investigations show that *Gambusia* takes a wide variety of food in its environment; e.g., aquatic insects including mosquitoes, Diatomeae Chlorophyceae and others floating on the water surface. Those results were similar to the results reported by Sato *et al.* (1972).

As a result of this program of releasing *Gambusia*, *Cx. tritaeniorhynchus* larvae were remarkably reduced in swamps and fallow rice fields by the early 1970's, and Japanese encephalitis was eradicated from Tokushima City. Since *Gambusia* can maintain its population even though there are no mosquito larvae, it is clear that *Gambusia* is a good potential agent for mosquito control. *Gambusia* is evidently a superior to *Oryzias* in competing for habitat under usual circumstances in the field. If we can keep such situations as in Kita-

Okinosu, where *Gambusia* spread quickly from their breeding places to swampy areas, obtaining high densities (Sato *et al.*, 1972), the scope for mosquito control that does not rely on insecticides would be broadened.

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カダヤシとメダカにおける脳炎媒介蚊の駆除,
および両種の蚊幼虫捕食能と競争
に関する生態学的研究

佐藤 英毅

1969年に徳島市に移殖されたカダヤシ *Gambusia affinis* の、蚊幼虫防除に果たす役割について調査した。繁殖地の池や溝で採集したカダヤシを、低湿地帯や下水溝に繰り返し放魚したところ、翌1970年には7カ所、1971年には36カ所、1972年には76カ所、および1973年には104カ所の水域に定着した。これらのうち、かつてメダカと蚊幼虫が共存していた水域が27カ所あった。これにカダヤシが定着し、1973年迄には、それらの全ての水域から蚊幼虫が消失した。一方、これらの水域ではメダカの生息密度が低くなった。カダヤシの生息密度が高かったのは、止水域ないし家庭排水が流入して富栄養化の進行している水域であった。カダヤシとメダカの混生する水域では、両種の生息密度が1 m²当たり400-500尾の範囲にあり、メダカの生息密度が既にこの範囲にある水域にカダヤシを定着させるためには、多量に放す必要があることが示唆された。室内試験の結果、カダヤシはメダカに比べて、蚊幼虫をよく捕食することが分かった。カダヤシとメダカを同一の容器で飼育した場合、カダヤシはメダカを攻撃し、駆逐してしまった。野生個体の胃内容物は類似し、両種とも広食性であるが、カダヤシの方が、より動物食性の傾向を示した。また、両種は空間および食物連鎖上の、競争種であることが示された。この研究では、蚊幼虫の防除にはメダカに比べてカダヤシの方が適していることが示された。ここで観察した水域では、カダヤシはメダカより生存競争上優位であり、その生息域を拡大している事が分かった。

A FIELD TEST OF 2-[1-METHYL-2-(4-PHENOXYPHENOXY) ETHOXY] PYRIDINE AGAINST PRINCIPAL VECTORS OF MALARIA IN A FOOT-HILL AREA IN THAILAND

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Abstract: An insect growth regulator, S-31183, 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine, was applied into 2 slow running streams to evaluate its efficacy against malaria vectors from the beginning of February to the end of April 1987. The evaluation of the efficacy was based on bioassay with the waters sampled from the streams and on the parity of the mosquitoes collected from the study site. The application of S-31183 induced almost 100% inhibition of emergence of *Anopheles maculatus* and *An. minimus*, 24 hr after application. The emergence was inhibited by averages of 80% in *An. minimus* and 56.7% in *An. maculatus*, in the end of the 4th week. Likewise, the nulliparous rates also decreased till the end of the 8th week.

INTRODUCTION

A number of efforts have been made to develop new compounds that are effective in the malaria vector control, due to insecticide resistance of the target insects and side effects to non-target organisms. Among these, attention is drawn to a new group of insecticide, the insect growth regulator (IGR). They interfere with the development of immature stages, resulting in prevention of both pupation and adult emergence. With respect to the development of resistance in insects against such chemicals, there were some reports indicating resistance development (Cerf and Georghiou, 1972; Dyte, 1972).

During the past decade, these synthetic IGRs have been studied extensively under laboratory and field conditions against insects of public health importance. A number of IGRs have been evaluated against mosquito larvae and proved to be quite effective to inhibit emergence (Jacob and Schoof, 1971, 1972; Schaefer and Wilder, 1972; Mulla *et al.*, 1974). Methoprene and diflubenzuron have been cleared for use in mosquito control program (Estrada and Mulla, 1986). Laboratory as well as field studies of methoprene demonstrated the promising control of many species of mosquitoes (Schaefer and Wilder, 1972; Hsieh and Steelman, 1974; Rathburn and Boike, 1975; Dame *et al.*, 1976; Ikemoto *et al.*, 1986; Ishii *et al.*, 1986; Sjogren

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et al., 1986). Diflubenzuron also offered a good potential for the control of mosquitoes breeding in diverse habitats (Mulla *et al.*, 1974, 1975; Mulla and Darwazeh, 1975, 1976; El Safi and Haridi, 1986). Recently, other types of juvenoid including S-31183 were introduced for evaluation against mosquitoes, *Aedes aegypti*, *Anopheles quadrimaculatus* and *Culex quinquefasciatus* by Estrada and Mulla (1986) and Mulla *et al.* (1986), and *Cx. tarsalis*, *Ae. melanimon* and *Ae. nigromaculis* by Mulla *et al.* (1986). Those field studies of S-31183 were mostly performed in ponds, pools, marshes or ditches with stagnant water. The evaluation in streams, which are breeding places of some important vectors of malaria in Thailand, has not been performed. Malaria control by residual insecticide did not accomplish effective results yet, though in addition to DDT residual spray, the larval control by both larvivorous fish (*Poecilia reticulata*) and a larvicide (such as Temephos) have been applied into these breeding streams by the Thai Government (1985).

In order to overcome the above mentioned problems the present study embarked to evaluate the activity in inhibition of emergence of the compound in slow running streams, the breeding habitat of 2 principal anopheline vector species of malaria, *Anopheles minimus* and *An. maculatus* in Thailand. The assessment of activity of this compound and the development of application method will provide the useful informations on the control of mosquitoes breeding in streams.

MATERIAL AND METHOD

Compound

S-31183, 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine, 0.5 G formulation provided by Sumitomo Chemical Co. Ltd. was used in this study. In order to have an information on the release rate of the IGR, 5 mg of the granule had been placed in 5 l of deionized water in a glass jar of 25 cm in diameter and 30 cm in height. It was kept at $25 \pm 1^\circ\text{C}$ for 24 hr. All the granules were removed by a pipette to filter paper in glass funnel then left at $25 \pm 1^\circ\text{C}$ overnight for drying. The dried granules were collected by a small brush and weighed. By this method the lost weight of the compound was proved to be 0.1 mg, which indicated the released compound within 24 hr in that container.

Preparation and Application of the Compound

A muslin cotton bag (20×15 cm) containing the required amount of the compound was fixed to the bottom of the stream at the application points, A₀ and B₀ (Fig. 1). The amount of the compound to be applied was estimated as follows: The widths of water surface (x) and

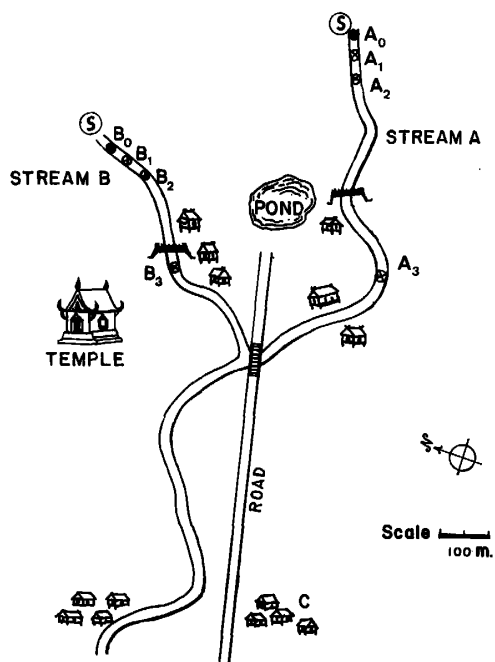


Figure 1 Experimental streams for S-31183 application (Wat tham pra pothisat, Tabkwang, Saraburi)

- application point
- ⊗ check point
- A₁, A₂ and A₃=50, 120 and 530 m from A₀
- B₁, B₂ and B₃=50, 20 and 270 m from B₀
- C = station for mosquito collection
- Ⓢ = spring

bottom (y), depth (h) and velocity of the running water per second (v) were estimated in both the streams (A and B). Therefore, the discharge (a) of water per second was calculated as,

$$a = (x + y) hv / 2$$

thus the amount (a) of the running water for 24 hr at A₃ and B₃ were 213,840 and 446,680 l, respectively. Because the formulation of the compound was 0.5%, 213.8 g was required in A stream and 446.7 g in B as shown in Table 1, for a concentration of 5 ppb.

Insects

Female *An. minimus* and *An. maculatus* were collected from the study site. The collection and maintenance method was the same as that one described by Kanda (1979). The mosquito maintenance was carried out till larval stage of the progeny for bioassay study, in the insectarium of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University.

Study Site

The field tests were conducted at a basin with 2 small streams in Tab-Kwang District, Saraburi Province, 130 km northeast of Bangkok, Thailand during February to April, 1987. The streams were designated as A and B (Fig. 1). The total length of both streams were approximately 1 km in A, and 0.55 km in B. They originated from 2 springs in hills and flowed down near a hamlet of approximately 10 houses. *An. minimus* and *An. maculatus* were found breeding in both the streams. These streams were mostly shaded by various species of tropical trees and with some bushes along both banks. The water was clear and clean with sandy substrate and very few grass vegetations. The water level was fluctuated among seasons, and the streams became dry up in late dry season at points about 15 m downstream from A₃ and B₃.

In February, 1987 the water temperature was 25-26°C with pH 7.0. The detailed hydrological data was presented in Table 1. Besides this basin we did not find any breeding site of the vectors around the village C (Fig. 1).

Assessment of Activity

Water was sampled for bioassay weekly in the first month after application and monthly thereafter. The evaluation was also performed by the density of female mosquitoes and their parity.

Parity Determination

Mosquito collections were performed by a cattle bait method in an orchard forest near the stream, indicated as C in Figure 1. Approximately 10 houses were scattered along the

Table 1 Hydrological data and the amount of S-31183 applied at the study site

Stream	Width (cm)		Depth (h) (cm)	Velocity of running water (v) (cm/sec)	Discharge (a) (l /day)	Amount of S-31183 (g) *
	Surface (x)	Bottom (y)				
A (A ₃)	155	70	8	2.75	213,840,000	213.8
B (B ₃)	260	140	11	2.35	446,680,000	446.7

A₃ and B₃ : Check points, see Fig. 1.

* : See the text.

streams where human bait collections were performed also. The collected *An. minimus* and *An. maculatus* were dissected to examine their ovariole relics following the method described by Detinova (1962).

Bioassay

Two-hundred and fifty milliliter of water sampled from the study site was put in an enamel bowl with 10 cm diameter and 8 cm height. Twenty-five of late 4th instar larvae were introduced to the bowls and fed daily with a small piece of artificial diet (Kanda, 1979). The mortality was examined everyday for 6 days or till all the adults emerged. The efficacy of the compound was expressed as per cent inhibition of emergence (%EI). Control tests were carried out in deionized water by the same method. Two replications were at least conducted for each water sample.

Confirmation of the Activity of the Compound in the Streams

Because the concentration of the chemical applied in the stream was not known, the activity was tested by serial dilution of water sampled. In the bioassay, 250 ml of water was sampled at 24 hr, 2 weeks and 4 weeks after application, and diluted with distilled water by $\times 5$, $\times 10$, $\times 20$, $\times 40$, $\times 80$, and $\times 200$. The larvae of *An. maculatus* were exposed to these solution by the same method described above. The similar tests were conducted with the control solution (5 ppb) of S-31183 which had been prepared and kept under laboratory condition. The test was performed at the same intervals.

RESULTS

Bioassay of the Water 24 hr after the Application

The activity of the compound, S-31183 was tested by bioassay method as follows: Emergence inhibition rates were tested with the water collected from various check points at 24th hr after the application of the compound. The results indicated any significant difference

Table 2 Inhibition rates by the compound (S-31183) of adult emergence of *An. minimus* and *An. maculatus* in the 2 running streams 24 hr after application

Check points	Distance from application points (m)	Inhibition rate of emergence	
		<i>An. minimus</i>	<i>An. maculatus</i>
Stream A			
A ₀ *	0	100	92.0
A ₁	50	98.9	96.2
A ₂	120	95.9	96.3
A ₃	530	98.0	100
Stream B			
B ₀ *	0	100	100
B ₁	50	98.9	100
B ₂	120	97.0	96.0
B ₃	270	97.9	96.1
Control	—	11.5	13.0

* : Application point. Control : Deionized water.

Table 3 Inhibition rates by the compound (S-31183) of adult emergence of *An. minimus* in the 2 running streams

No. of weeks after application	Stream A			Stream B			Control
	A ₁	A ₂	A ₃	B ₁	B ₂	B ₃	
0 *	14.2 (98)	13.1 (99)	14.7(101)	15.0(100)	14.2 (98)	13.8(101)	10.2(107)
1	100 (108)	97.0(101)	98.9 (95)	99.0(103)	98.9 (98)	88.5(104)	12.0(100)
2	—	—	—	—	—	—	—
3	100 (102)	86.1(108)	91.1 (90)	83.7 (92)	87.1(101)	93.0(100)	9.7(103)
4	100 (99)	88.0(100)	84.2 (95)	45.9 (98)	91.9 (99)	70.0(100)	11.1(108)
8	25.5(102)	32.0(100)	22.2 (99)	20.2 (94)	35.6 (90)	22.0(100)	10.5(104)

() : Number of larvae tested. * : The water was sampled before the application.

Table 4 Inhibition rates by the compound (S-31183) of adult emergence of *An. maculatus* in the two running streams

No. of weeks after application	Stream A			Stream B			Control
	A ₁	A ₂	A ₃	B ₁	B ₂	B ₃	
0 *	15.5(103)	13.9(101)	15.1 (99)	14.7(101)	16.0(100)	14.0(100)	10.7(103)
1	80.8(104)	80.0(100)	96.0(100)	78.5(104)	87.7 (98)	84.6(104)	11.2 (98)
2	82.7 (98)	87.2 (94)	93.3 (90)	97.7(110)	96.9 (99)	82.2(101)	19.4 (98)
3	54.6(110)	86.4(114)	71.1 (90)	76.7(107)	82.1 (78)	76.3 (76)	18.3(104)
4	60.0(102)	53.5 (99)	65.2 (88)	37.3(102)	54.2 (96)	69.6 (92)	15.8(101)
8	33.3(108)	29.4(102)	20.0(105)	20.0(110)	20.6(102)	23.3(105)	10.3(104)

() : Number of tested larvae. * : The water was sampled before the application.

Table 5 Inhibition rates of adult emergence of *An. maculatus* exposed to variously diluted water sampled from one of check points, A₃

Dilution	Sampling time after application					
	24 hr		2 w		4 w	
× 1	96.0	(100)	93.3	(90)	65.2	(92)
× 5	95.2	(84)	92.9	(98)	51.8	(81)
× 10	94.5	(91)	90.2	(92)	51.4	(72)
× 20	93.1	(87)	87.6	(89)	34.2	(76)
× 40	88.4	(95)	81.4	(86)	34.7	(69)
× 80	89.0	(82)	83.3	(90)	35.4	(82)
× 200	84.5	(96)	69.5	(82)	—	—
Control	10.4	(96)	12.8	(86)	9.5	(95)

Control: Tested in deionized water. () : Number of larvae tested.

among those points. The rates were proved 92.0% for *An. maculatus* and 100% for *An. minimus* at A₀ (Table 2). Thus the water collected 24 hr after the application had high inhibitory effect on the adult emergence.

Bioassay of the Water Collected Weekly from the Study Site

Water from the applied streams was bioassayed weekly in the first month of application. Though the inhibition of adult emergence of *An. minimus* decreased gradually at every check point, more than 70% inhibition of emergence was retained in both streams till the end of the 4th week (Table 3). The inhibition rates were more than 20.0% for *An. minimus* and *An. maculatus* still in the end of the 8th week as Tables 3 and 4 show.

Confirmation of the Activity of the Compound Diluted to the Stream Water

In order to confirm the activity of the compound resolved in the water, the water sampled at the check point A₃ at various time periods after application was diluted 5 times ($\times 5$), $\times 10$, $\times 20$, $\times 40$, $\times 80$, and $\times 200$, and tested by bioassay method (Table 5). The inhibition rate of *An. maculatus* in the water collected was 84.5% at $\times 200$ 24 hr after application, and 83.3% at $\times 80$, and 69.5% at $\times 200$ in the end of the 2nd week. In the end of 4th weeks the diluted water at $\times 5$ and $\times 10$ demonstrated more than 50.0%, but at over $\times 20$ of dilution the rate was lower than 50% (Table 5). In order to reconfirm the activity of the test water, the solutions of the compound by similar diluting times from the concentration of 5 ppb were prepared and kept in the laboratory as the control of the applied stream water (Table 6).

Table 6 Mortality of larvae of *An. maculatus* exposed to variously diluted solution of 5 ppb S-31183 prepared in laboratory

Dilution	Conc. (ppb)	Time after preparation					
		24 hr		2 w		4 w	
$\times 1$	5.0	100	(96)	100	(90)	95.9	(98)
$\times 5$	1.0	100	(99)	100	(86)	94.0	(98)
$\times 10$	0.5	100	(100)	100	(91)	94.3	(87)
$\times 20$	0.25	97.9	(98)	97.8	(81)	76.5	(81)
$\times 40$	0.125	95.5	(89)	94.0	(84)	56.0	(91)
$\times 80$	0.0625	91.5	(82)	87.8	(90)	53.0	(83)
$\times 200$	0.025	90.0	(90)	83.9	(81)	40.5	(84)
—	Control	10.4	(96)	12.8	(86)	9.5	(95)

Control : Tested in deionized water. () : Number of larvae tested.

Table 7 Nulliparous rates of the 2 vector species collected periodically on a cattle bait at the point C after application

No. of weeks after treatment	<i>An. minimus</i>			<i>An. maculatus</i>		
	Density/b/n	No. examin.	Nullip. rate (%)	Density/b/n	No. examin.	Nulliparous rate (%)
Pre-app.	174	54	36.3	43	43	52.9
1	11	25	27.3	—	—	—
2	90	50	15.9	12	28	33.3
3	101	50	7.9	19	35	14.3
4	45	45	9.1	—	—	—
8	22	40	10.5	10	25	25.0
12	12	30	41.7	26	50	64.0

Density/b/n: Mosquito density per 1 cattle per night.

The results showed stronger inhibition activity than in Table 5.

Even if the water in the streams increase, the inhibition activity might be retained up to $\times 20$ dilution.

Assessment by Parity of Females Collected by Bait

The parous rate of the vectors and their density were investigated to confirm the efficacy of the compound. The results are shown in Table 7. Before the application of the compound the nulliparous rate was 36.3% for *An. minimus* and 52.9% for *An. maculatus*. In *An. minimus*, it gradually decreased after the application from 27.3% in the end of the 1st week to 7.9% in the end of the 3rd week. The low parous rate combined till approximately the 8th week. The rate, however, increased up to 41.7%, which was similar to the rate before the application, in the end of the 12th week. Also in *An. maculatus*, the nulliparous rate gradually decreased and indicated the lowest of 14.3% in the end of the 3rd week, and it recovered to the pre-application level by the end of the 12th week.

DISCUSSION

The insect growth regulator was tested for the first time by the present study against vectors breeding in running water. The compound used in this study was found very effective even at extremely low concentrations. Iwanaga and Kanda (1988) reported that the LC_{50} of this compound against *An. balabacensis* was 0.04 ppb in the laboratory. Therefore, the concentration of 5 ppb was supposed to be enough to inhibit the emergence of the two species, *An. minimus* and *An. maculatus* in this field application. The amount of the chemical applied was expected to produce 5 ppb concentration, which was 100 times higher than the LC_{50} against *An. balabacensis* (Iwanaga and Kanda, 1988). Amalraj *et al.* (1988) induced 100% mortality with complete inhibition of emergence at 1 ppb of the compound on *An. stephensi*. The concentration of 5 ppb was much lower than in the field application of Temephos larvicide against *An. minimus* in Thailand, which was 0.5 ppm (Phatipongse, 1983) and 1.0 ppm (Malaria Division, 1985).

The compound was applied at the dosage of 213.8 g and 446.7 g into the stream A and B, respectively. These amounts were derived from the water discharge in 24 hr at A_3 and B_3 , and the formulation of the chemical (0.5% granule). The dissolving velocity of the chemical in stagnant water was 1/50 per 24 hr as estimated earlier. This suggests that 50 days would be needed for complete dissolving. Therefore, the effective concentration of the chemical could be estimated as 0.1 ppb and should last for 50 days. The present results for the inhibition of adult emergence demonstrated the efficacy of about 70% till the end of 4th week in the field application. This shortening of the duration in the treated water of the streams was interpreted to be due to the loss of the compound by running water, and the faster dissolving rate than in stagnant water. From subsequent observations, the inhibition rate of the compound gradually decreased to 20-35.6% in *An. minimus* and 23.3-33.3% in *An. maculatus* by the 8th week (Tables 3 and 4). If 70% inhibition of emergence was considered as an effective control level (Ishii *et al.*, 1987), the effective duration of the compound was 4 weeks against the 2 vectors. The effectiveness of this compound in field tests was reported by many authors. Mulla *et al.* (1986) obtained 85-92% inhibition of emergence of *Cx. tarsalis* by 7 day after treatment with 0.005-0.01 lb/acre of S-31183. Whereas Amairaj *et al.* (1988)

reported 100% inhibition of emergence of *Cx. quinquefasciatus* in cesspits for 7 days by the application of this compound at the dosage of 0.05 mg/l.

The larval control programme of malaria vectors in Thailand was conducted in some areas by releasing of larvivorous fish (*Poecilia reticulata*) and also by application of 1 ppm of Temephos. An effective control against *An. minimus* was obtained from the latter but without residual effect (Annual Report, Malaria Division, 1985). Phatipongse (1983) tried 0.5 ppm of Temephos in a stream to control *An. minimus* but did not study the residual toxicity. The present study suggested that S-31183 can be used as a larvicide in running streams, since it exhibited a prolong activity and was effective at lower dose. Moreover, the IGRs are recommended for mosquito control because of little effects on non-target organisms (Mulla *et al.*, 1975). Additionally, most IGRs are known to have low mammalian toxicity and relative safety to fish and wild life in the breeding place as reported by Mulla *et al.* (1986).

Referring to the present results, it was of interest to note that in rainy season the streams were full up with water and continuously flowing with somewhat higher speed. The application of the compound might induce a different pattern in inhibition of emergence in dry season. Even in the period when nulliparous rate was suppressed, the malaria transmission could occur to a small extent by parous females. The effective control can probably be achieved if other adult control methods were integrated.

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WATER DEMAND AND SCHISTOSOMIASIS AMONG THE GUMAU PEOPLE OF BAUCHI STATE, NIGERIA

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Abstract: A helminthological study of stool and urine samples collected from 1,037 people from four rural communities in Gumau District of Bauchi State, Nigeria was carried out using formo-ether concentration and direct centrifugation methods respectively. The study is the first parasitological survey to be carried out in the district. Thirty-nine per cent of examined persons were infected. Ova of *Ascaris lumbricoides*, *Schistosoma haematobium*, *Schistosoma mansoni* and *Trichuris trichiura* were most common. Hookworm, tapeworm and *Strongyloides stercoralis* were also encountered, 17.9% and 10.8% of examined persons had *S. haematobium* and *S. mansoni* infections respectively. Water demand index (number of persons per well) for each community was calculated as a ratio of the total population to the number of safe water sources available in that community. A significant relationship was found between water demand index and *S. haematobium* ($r=0.95$) on one hand, and *S. mansoni* ($r=0.88$) on the other ($P<0.01$). Such relationship was not found in soil borne helminth infections. A similarly significant association was found between distance from river and prevalence of *S. haematobium* ($r=0.94$) on one hand and *S. mansoni* ($r=0.95$) on the other ($P<0.01$) but not found in soil-borne helminth infections. These findings seem to suggest that water demand index may be an important factor in the epidemiology of Schistosome infections in rural communities. Water demand index may also be a useful field technique for estimating and comparing the rates of water associated helminth infections in rural communities with comparable environmental conditions.

INTRODUCTION

The source of drinking water is regarded as one of the most important epidemiological factors in helminth infections. It has been suggested that the provision of potable water is a possible solution to the problem of helminthiasis (Gilles, 1964; Cowper, 1967; Akoh, 1980). This suggestion is based on the fact that human activities are associated with helminth infections and that prevalence of helminthiasis is a measure of the extent to which the source of water is contaminated with waste. However, there are indications that the provision of water alone is not enough to combat the increasing rate of water-associated helminthiasis, especially in rural areas where the demand for potable water needs to match its supply to an ever rising population.

MATERIALS AND METHODS

Description of study area: The Gumau district of Bauchi State lies between 1,000 and 1,300 m above sea level in the northern Guinea Savannah of Nigeria (Figure 1).

The annual rainfall ranges between 1,270 and 1,828 mm while the annual temperature ranges between 20°C and 30°C (Kowal and Knabe, 1972). The studied communities are situated at the following locations:

1. Gumau (10°15'N 9°06'E)
2. Laru (10°03'N 9°16'E)
3. Badiko (10°09'N 9°13'E)
4. Magama (10°02'N 9°17'E)

A great deal of human activity (bathing, laundry and fishing) takes place along rivers Delimi, Badiko and Bindiri at specific sites which retain water throughout the year.

Methods: Procedures for the collection and examination of stool and urine specimens were those described by King (1972). The population estimate of each community was obtained from the district office which keeps a record of tax-paying adults, birth and death and of school enrolment. The number of safe water sources was obtained by personally counting them with the assistance of the district Clerk and the Social Welfare Officer. The main criteria for determining safe wells were

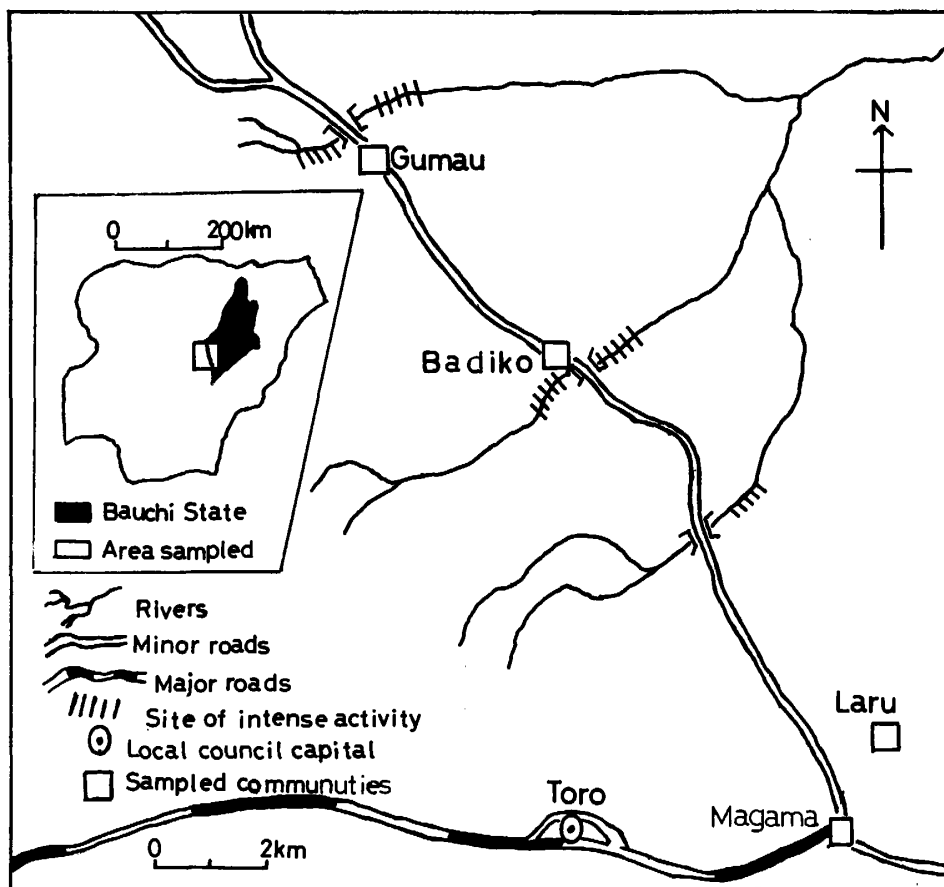


Figure 1 Map of study area: Gumau District, Bauchi State.

- a) depth must be at least 7.5 m
- b) there must be an extension slab at the mouth end which must be 0.5 m above ground level, and
- c) the well must have a metal or wooden cover. These criteria are the approved standards by the Local Government Council.

The water demand index (the number of persons per well for each community) was calculated as a ratio of the total population to the number of safe water sources in that community. The distance from the village centre to the nearest major alternative water source was determined.

Data analysis: Simple Chi-square test and Friedman's two-way ANOVA by ranks were used to analyze the results for heterogeneity and significant variations.

RESULTS

The water demand index of each community is shown in Table 1, Table 2 shows the prevalence and distribution of each helminth in each of the four communities. *S. haematobium* and *S. mansoni* were the only species of Schistosomes that were encountered. The prevalence of *S. haematobium* was analyzed separately but included in Table 2. Distribution of prevalence of infection does not seem to be even ($\chi^2 P < 0.01$). The distances from the village centre to the nearest major alternative water source are shown (Table 2).

The water demand index differed between villages. There seemed to be a significant association between water demand index and *S. haematobium* prevalence ($r=0.95$, t-test, $P < 0.01$) on one hand and *S. mansoni* ($r=0.95$, t-test $P < 0.01$) on the other (Figure 2). An association was also observed between *Taenia* sp. and water demand index ($r=0.70$, t-test, $P < 0.05$) but not in any other helminth.

A similarly significant association seems to exist between the distance from river and *S. haematobium* ($r = -0.94$, t-test $P < 0.01$) on one hand and *S. mansoni* ($r = -0.88$, t-test, $P < 0.01$) on the other. Such an association was not observed between distance from river and any other helminth.

DISCUSSION

The type of association between *Ascaris* infection and water which was suggested by Bidinger *et al.* (1981) was not observed in this study. The remarkable association between

Table 1 Water demand index of each community

Community	Nearest river	Population estimate	No. of wells	No. of persons per well*
Gumau	Delimi	10,000	26	384.6
Badiko	Badiko	4,900	14	350.0
Laru	Bindiri	2,000	7	285.7
Magama	Bindiri	3,800	32	118.8
Total		20,700	79	262.0

*: Water demand index.

Table 2 Distribution of each helminth in the district *

Helminth	Gumau Inf. (%)	Badiko Inf. (%)	Laru Inf. (%)	Magama Inf. (%)	Total Inf. (%)	Water demand and parasite	River distance and parasite
<i>S. haematobium</i> †	125 (24.0)	38 (18.0)	14 (9.5)	4 (2.6)	181 (17.5)	§	
<i>A. lumbricoides</i>	112 (21.5)	51 (23.9)	41 (27.7)	29 (18.7)	233 (2.5)	ns	ns
<i>S. mansoni</i>	80 (15.4)	23 (10.8)	6 (4.0)	3 (1.9)	112 (10.8)	§	
Hookworm	17 (3.3)	18 (8.5)	6 (4.0)	5 (3.2)	46 (4.4)	ns	ns
<i>Taenia</i> sp.	6 (1.2)	2 (0.9)	2 (1.4)	5 (3.2)	15 (1.4)	‡	ns
<i>H. nana</i>	4 (0.8)	7 (3.3)	3 (2.0)	3 (1.9)	17 (1.6)	ns	ns
<i>T. trichiura</i>	4 (0.8)	3 (1.4)	9 (6.1)	—	16 (1.5)	ns	ns
<i>S. stercoralis</i>	4 (0.8)	—	—	—	4 (0.4)	ns	ns
No. Exam.	521	213	148	155	1,037		
Prevalence (%)	39.9	46.0	41.2	24.5	39.9		
Water demand index	384.6	350.0	285.7	188.8	262.0		
Distance from nearest river (m)	600	410	5,000	6,000			

*: These figures include mixed infection. †: Urinary Schistosomiasis was analyzed separately.

‡: $P < 0.05$ §: $P < 0.01$ ||: negative correlation.

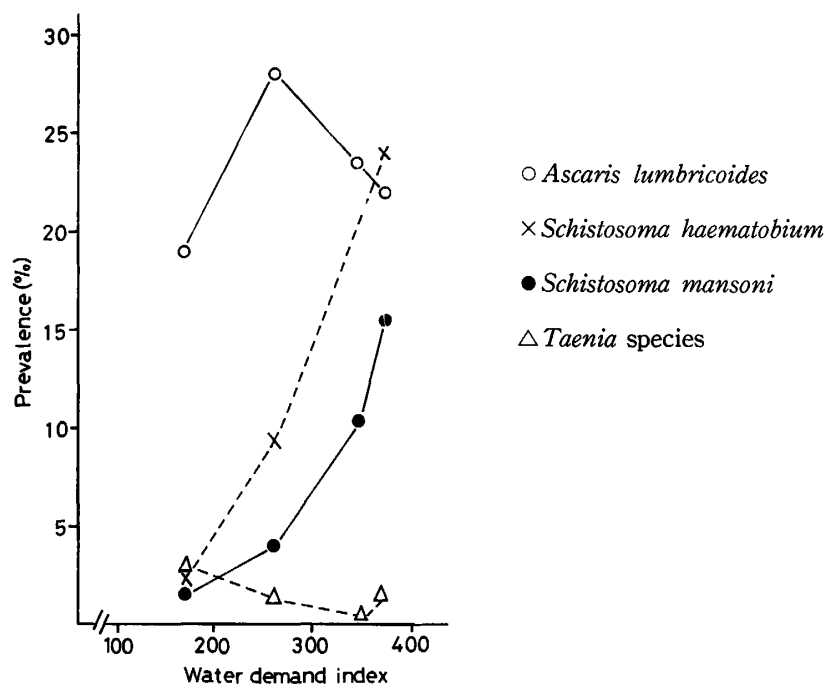


Figure 2 Prevalence of some helminths and water demand index.

water demand index and Schistosome infection in the district is self evident. The results suggest that the rate of Schistosome infections tend to increase with increase in water demand index. The explanation for these observations may be due to the contamination of alternative sources of water. This may lead to an increase in the number of Schistosome intermediate hosts which become infected as a result of contamination. The number of cercariae that are released by the intermediate hosts increases thus increasing the chances of human-parasite contact and so enhancing a subsequent rise in the prevalence of disease among the human population who in turn further contaminate the alternative water source through continual contact. Water demand index may be a major factor in the transmission of water-associated helminths. The association between *Taenia* sp. (which has no direct association with water) is not well understood and may likely be due to sampling error.

It is literarily possible to have almost the entire community infected as a result of the association between water demand, the environment and Schistosomes. This explanation is especially likely since the distances from the rivers seem to have a negative association with only *S. haematobium* and *S. mansoni* infections in this study. This may be due to human behaviour; the easier the access to the alternative source, the more the number of people who may contaminate it in a community with large water demand index and the greater the chances of contact with infective stage of Schistosomes. The distance from river does not seem to affect the prevalence of other helminths in this study.

Although the findings of the present study may not be conclusive regarding the relationship between water demand of a community and its rate of Schistosome infections, the results do reveal an interesting association between them. This association may be a useful field technique for estimating and comparing the rate of Schistosome infections in communities with similar environmental conditions.

ACKNOWLEDGEMENT

This study is part of a research project which was supervised by Dr. V.N. Okwuosa to whom I am very grateful for useful advice. The assistance of the Gumau District Office is gratefully appreciated.

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DESCRIPTION OF A NEW SPECIES OF THE BLACK
FLY SUBGENUS *SIMULIUM* (*ECTEMNASPIS*)
FROM THE ANDES OF ECUADOR (DIPTERA:
SIMULIIDAE)

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Received January 13 1989/Accepted April 17 1989

Abstract: *Simulium* (*Ectemnaspis*) *pautense* sp. n. was described based on female, male, pupal and larval specimens collected in small streams of Andean region in Ecuador. This new species is close to *S. (E.) gabaldoni* Ramírez Pérez from Venezuela by having the 6-filamented pupal gills, but differs in the female from the latter species by lacking denticles in the cibarium and by the coloration of the legs.

INTRODUCTION

Coscarón (1984) revised a neotropical simuliid fauna, and assigned 37 black fly species into the subgenus *Simulium* (*Ectemnaspis*) Enderlein. Of these, eight species have been reported from Ecuador (*S. ignescens* Roubaud, *S. lutzianum* Pinto, *S. lewisi* Ramírez Pérez, *S. pseudoantillarum* Ramírez Pérez and Vulcano, *S. dinelii* (Joan), *S. shewellianum* Coscarón and *S. bicoloratum* Malloch, *S. romanai* Wygodzinsky (e.g., Coscarón, 1984; Takaoka and Hirai, 1987).

Recently, an additional undescribed species of this subgenus was collected from the high Andes of Ecuador. This bears a combination of the following features: the adult scutum and abdomen colored partially yellowish, subtriangular paraprocts, female claws with a small subbasal tooth, and short, subcylindrical male style. All these fit the definition of the subgenus *Simulium* (*Ectemnaspis*), given by Coscarón (1984).

In this paper, this is below described as a new species.

DESCRIPTION

Simulium (*Ectemnaspis*) *pautense* sp. n.

Female. Wing length 2.4-2.6 mm. Head brown; proboscis, maxillary palps and antennae dark brown to black. Frons and clypeus with silvery pollinosity. Scutum orange, ornamented as in Figure 1, and greenish yellow laterally. Scutellum greenish yellow. Postscutellum velvet black with 1+1 small greyish patches. Pleura mostly pale yellow with a small elongated brown stripe over the pleural membrane, and brown basally. Wing hairs brown. Legs dark brown with concolored hairs. Abdominal tergites I-IV yellow, with tergal plates III and IV

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pale brown; tergal plates V-IX blackish brown, waxy bright except plate V velvet bright (Fig. 2). Frons convergent below (Fig. 3); frontal angle 78 degrees. Frontoocular triangle as wide as high (Fig. 4). Mandible with 29-33 teeth. Maxilla with 24 or 25 teeth. Sensory organ of maxillary palp shorter than 1/2 of basal segment. Basal portion of cibarium with reinforced border, and without denticles (Fig. 5). Basal section of R bare. Sternite VIII with darker middle area, and with about 27 hairs on each side; gonapophyses reinforced on the internal border (Fig. 6); paraproct about as long as wide at base (Fig. 7); cercus with straight border; genital fork with middle branches heavily sclerotized (Fig. 8).

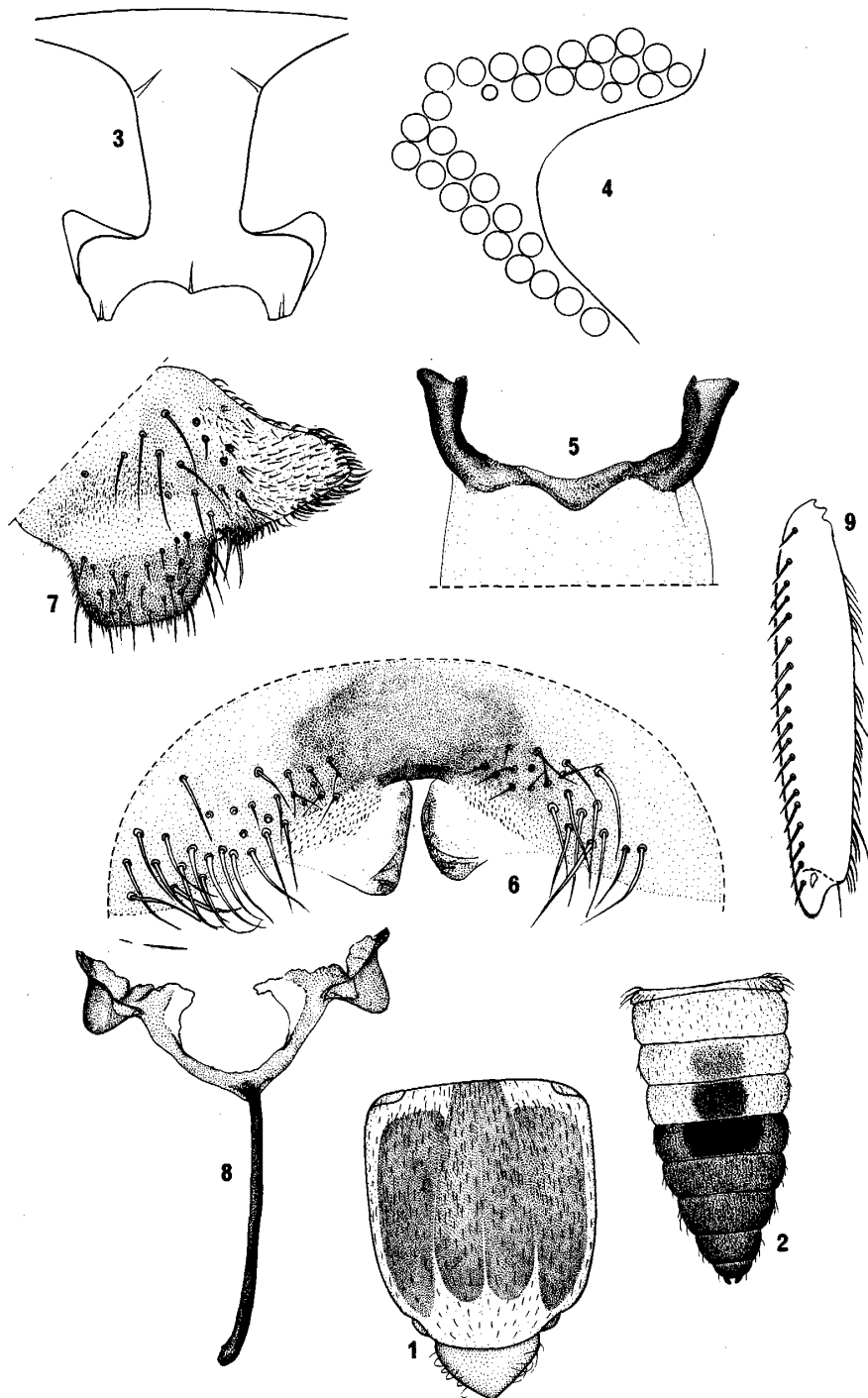
Male. Wing length 2.3-2.5 mm. Coloration very close to female. Scutum pale but yellow orange on the top, and with a T-formed black stripe on the anterior third and a pair of black, broad longitudinal stripes sublaterally (Fig. 10). Hind basitarsus about 5× as long as its width (Fig. 9). Style about 4/5× length of coxite (Fig. 11). Median sclerite elongated with a distal concavity. Parameres with several stout hooks (Fig. 13).

Pupa. Gill (Figs. 14 and 15) with 6 slender filaments and 2.7-3.3 mm long. Frontoclypeus and exposed portion of thorax with rounded and smooth tubercles (Figs. 16 and 17). Trichomes on frontoclypeus and thorax curved basally and divided into 3-6 branches (Figs. 15 and 16). Cocoon (Fig. 14) simple slipper-shaped, thickly woven with evident threads; its length 3.0-3.3 mm at base and 2.4-2.8 mm dorsally.

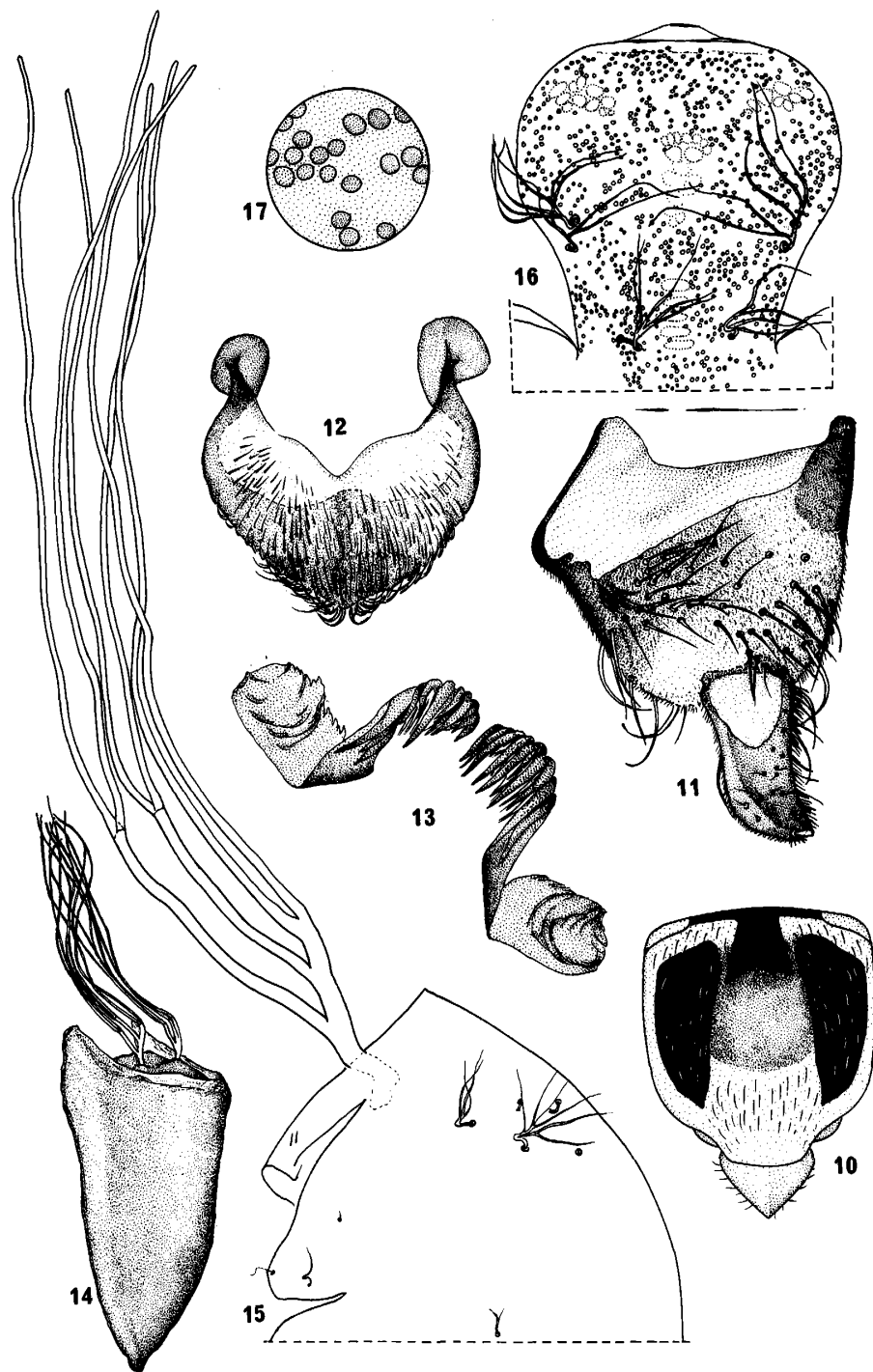
Mature larva. Body shaped as in Figure 18, and 5.5-5.8 mm long. Head brown with negative head spots (Fig. 19); cephalic capsule 0.55 mm in maximum width. Hypostomium with median tooth as high as corner teeth and with 5 setae on each side (Fig. 20). Postgenal cleft deep and narrow (Fig. 20). Antenna (Fig. 21) shorter than base of cephalic fan; relative length of segments 1-3, 1.0:1.0-1.2:1.6-2.0. Cephalic fan with 42-48 rays. Mandible with 8-10 internal teeth disposed in 2-3 rows (Fig. 22). Thorax and abdomen mostly greenish and their cuticles bare except setose portion near anal sclerite. Proleg sclerite comb with 23-25 teeth (Fig. 23). Anal sclerite shaped as in Figure 24, and with several setae on each side. Posterior circlet with 73-76 rows of 14-15 hooks. Anal gill with 12 secondary lobules on each lobe.

Material examined. Holotype female, reared from pupa, was collected from a small stream about 20 cm wide (ca. 2,500 m in altitude) in Paute, Azuay, Ecuador, 5 July, 1988, by H. Takaoka; allotype male, paratypes 2 females and 1 male, 10 pupae and 2 larvae, same data as holotype. Paratypes 8 females and 5 males, reared from pupae, were collected from a small shallow stream 10-30 cm wide (ca. 3,000 m in alt.) in Cuenca, Ecuador, 4 July, 1988, by H. Takaoka. All the type specimens are deposited in Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina.

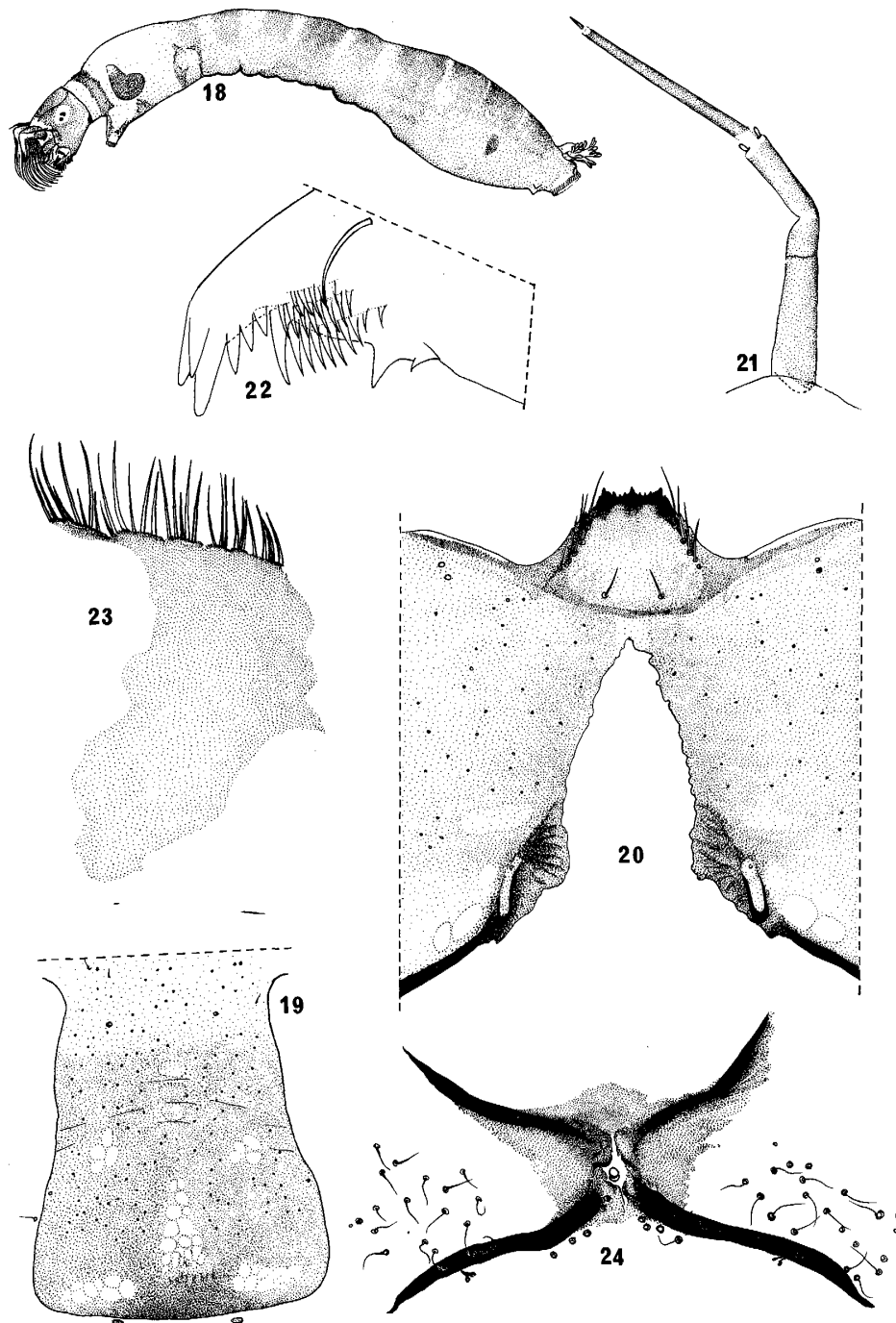
Remarks. *Simulium pautense* sp. n. is close to *S. gabaldoni* reported from Venezuela (Ramírez Pérez, 1971) by having the 6 respiratory filaments in the pupa, but differs in the female from the latter species by the absence of tubercles in the cibarium and by the entire darkness in the legs.



Figs. 1-9. *Simulium pautenese* sp. n. 1-8, female and 9, male. 1, scutum and scutellum in dorsal view; 2, abdomen in dorsal view; 3, frons; 4, fronto-ocular triangle; 5, cibarium; 6, sternite VIII and anterior gonapophyses; 7, paraproct and cercus in lateral view; 8, genital fork; 9, hind basitarsus.



Figs. 10-17 *Simulium pautense* sp. n. 10-13, male and 14-17, pupa. 10, Scutum and scutellum in dorsal view; 11, coxite and style in dorsal view; 12, ventral plate in frontal and ventral view; 13, parameres; 14, pupa and its cocoon; 15, thoracic integument and respiratory filaments; 16, frontoclypeus with tubercles and trichomes; 17, round and smooth tubercles on frontoclypeus.



Figs. 18-24 *Simulium pautense* sp. n. larva. 18, lateral view of larva; 19, cephalic apotome; 20, ventral view of head capsule showing hypostomium and postgenal cleft; 21, antenna; 22, apical tip of mandible; 23, sclerite comb of proleg; 24, anal sclerite.

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エクアドルのアンデス高地で見いだされた *Ectemnaspis* 亜属の
1 新種ブユの記載

Sixto Coscarón¹・高岡 宏行²

エクアドルのアンデス高地のパウテ町において採集されたブユの成虫，蛹および幼虫を分類学的に検討した結果，*Simulium* 属の *Ectemnaspis* 亜属に属する新種であることが分かったので新種名を与え，記載を行った。

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EFFECT OF GASTROGRAFIN ON *DIPHYLLOBOTHRIUM LATUM*, *TAENIA SAGINATA* AND *DIPLOGONOPOLUS GRANDIS* INFECTION

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Abstract: The successful treatment of *Diphyllobothrium latum*, *Taenia saginata* and *Diplogonoporus grandis* infections with Gastrografin is herein reported. Eleven subjects (8 cases of *D. latum*, 2 of *T. saginata* and one of *D. grandis* infection) found to have tapeworm infection based on the history of discharge of gravid segments and/or positivity for tapeworm egg were first pretreated with barium enema procedure and were treated with 300 to 500 ml Gastrografin by duodenal manipulation. All cases who indicated the presence of the tapeworm as a radiolucent shadows were completely treated with Gastrografin. All tapeworms with scolex expelled were actively moving in the saline. The time of treatment (from 15 to 30 min) was shorter than that of other medical treatments. No side effects were observed. In the present study, the treatment with Gastrografin was found to be a satisfactory anthelmintic method against *D. latum*, *T. saginata* and *D. grandis* infection.

INTRODUCTION

The drugs such as bithionol (Yokogawa *et al.*, 1962), paromomycin sulfate (Salem and El-Allaf, 1969), mebendazole (Vandepitte and Thienpont, 1972), niclosamide (Kihara *et al.*, 1973) and praziquantel (Paz, 1977) have commonly been used as anthelmintics against human intestinal tapeworm infection. Although these drugs are very effective, the scolexes are usually not found in their feces after the treatment.

Recently, it has been shown that the Gastrografin (Shering), a water soluble contrast material of the gastrointestinal tract, has possessed an anthelmintic effect on intestinal tapeworm (Nakabayashi *et al.*, 1984; Waki *et al.*, 1986).

In this report, Gastrografin was used for treating *Diphyllobothrium latum* (*D. latum*), *Taenia saginata* (*T. saginata*) and *Diplogonoporus grandis* (*D. grandis*) infections and was most effective for removing the tapeworms.

MATERIALS AND METHODS

This study consisted of 11 subjects; 8 cases with *D. latum*, 2 with *T. saginata* and one with *D. grandis* infection. All subjects were confirmed to have intestinal tapeworm infection by

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the following two criteria: history of discharge of gravid segments and/or positivity for tapeworm eggs in their stools and their anus.

The treatment was performed according to the methods of Waki *et al.* (1986) with some modifications. The routine treatment was as follows: The day before the treatment, the patient had restricted food intake, for example, Besbion (Fujisawa Pharmaceutical Co., Ltd.) and Boncolon (Ohtsuka Chemical Co., Ltd.), instead of ordinary meal. Thereafter, 250 ml of magnesium citrate (Magcorol, Horii Pharmaceutical Indus., Co., Ltd.) and 4 tablets of senna (Adjust-A, Kowa Co., Ltd.) were given to the patient at 8.00 p.m. and 11.00 p.m., respectively. On the day of treatment, the patient was kept fast until the treatment but isotonic drink such as Pokalisweat (Ohtsuka Pharmaceutical Co., Ltd.) was made enough, for the prevention of dehydration. The patient was kept in the bed for X-ray fluoroscopy. A duodenal tube was inserted through the mouth until the tip reached the flexura duodeno-jejunalis. Gastrografin (300 ml) was injected through the tube with a plastic disposal syringe (50 ml, Terumo Co., Ltd.) for 5 min. Under fluoroscopic monitoring, the worm was found as a radiolucent shadow descending in the intestinal lumen treated with Gastrografin. When the peristalsis of the intestine did not occur vigorously, the adequate volume of Gastrografin was added. When the parasite reached the anal side through the descending colon, the patient was forced to defecate. The moving worm with scolex was discharged with the stool.

RESULTS

Table 1 summarizes the clinical profiles and treatment regimen of the 11 patients. Of the 11 cases with intestinal tapeworm infection included in this study, 9 (81.8%) were male and 2 (18.2%) were female. Their age was ranged from 13 to 55 (41.5 ± 10.9) years old. Using 300 to 500 ml Gastrografin, the entire tapeworm with scolex were discharged within 15 to 30 min. In all cases, the tapeworms were alive, unfragmented and moved actively in the saline.

Case 1, 2, 3, 5, 6, 8, 10 and 11 were *D. latum* infection occurred by eating raw fish (salmon and trout). Case 4 and 9 were *T. saginata* infection occurred by eating incompletely cooked beef meat. Case 7 was *D. grandis* infection, but the cause of this case was unknown.

Two cases (case 10 and 11) was not able to find out the tapeworm in X-ray monitor in the first treatment. In these cases, tapeworms were not discharged with their stool. But, 45 days after the treatment, the eggs of *D. latum* were detected from their feces by stool examinations. Two months after the first treatment, the second treatments were done. In these times, the tapeworms could be found out in X-ray monitor from both cases. These tapeworms were expelled with their stool and these cases were completely treated by the repeat of this method.

Figure 1 showed *T. saginata* (case 4) moving very actively in the ileo-caecalis portion and each proglottid of *T. saginata* was clearly described as a radiolucent region in this photograph. Figure 2 showed *D. latum* (case 5) revealing at the same region in Figure 1. Figure 3 showed the worm (*T. saginata*, case 4) recognized as a radiolucent region in the rectum before defecation. After this photograph, this worm was discharged through the anus (case 4). Figure 4 showed the entire shape of *T. saginata* (case 9). The scolex (arrow) of this worm was shown in this photograph.

In every case, the side effects of Gastrografin were not observed.

Table 1 Summary of the treatment with Gastrografin

Case number	Age Sex	Subjective symptoms	Gastrografin dosage	Total length of worm	Species of tapeworm
			Time required for treatment		
1	48 y M	soft stool	400 ml 25 min	736 cm	<i>D. latum</i>
2	42 y M	abdominal pain	400 ml 30 min	506 cm	<i>D. latum</i>
3	39 y M	abdominal discomfort	300 ml 25 min	475 cm	<i>D. latum</i>
4	46 y M	none	400 ml 20 min	661 cm	<i>T. saginata</i>
5	41 y F	abdominal discomfort	400 ml 15 min	450 cm	<i>D. latum</i>
6	48 y M	abdominal discomfort	500 ml 15 min	225 cm	<i>D. latum</i>
7	55 y M	tenesmus & abdominal discomfort	400 ml 15 min	273 cm	<i>D. grandis</i>
8	13 y F	abdominal discomfort	300 ml 15 min	708 cm	<i>D. latum</i>
9	42 y M	abdominal discomfort	500 ml 25 min	275 cm	<i>T. saginata</i>
10*	35 y M	soft stool & abdominal discomfort	450 ml 15 min	600 cm	<i>D. latum</i>
11*	47 y M	abdominal pain	340 ml 23 min	890 cm	<i>D. latum</i>

*The data of these cases (case 10 and 11) were showed from the second treatment.

DISCUSSION

For the last 30 years, the intestinal parasite infection such as ascariasis, hookworm diseases, trichuriasis and clonorchiasis has been markedly reduced in Japan.

However, intestinal tapeworm infection is widely distributed in Japan, because the Japanese likes to eat raw fish (sashimi) and incompletely cooked beef meat which are the second intermediate host of *D. latum*, *D. grandis* and *T. saginata*.

In foreign countries such as USA, the Japanese style eating at sashimi and sushi bar where such dishes are served, is becoming popular as healthy foods. And these foods has often been suggested as a contributing factor to the spread of fish tapeworm infections in foreign countries.

Nakabayashi *et al.* (1984) and Waki *et al.* (1986) reported that the Gastrografin was a useful drug against *T. saginata* and *D. latum* infection. In their reports, they concluded that some physical relation between the parasite and the intestine were important for expelling the tapeworm.

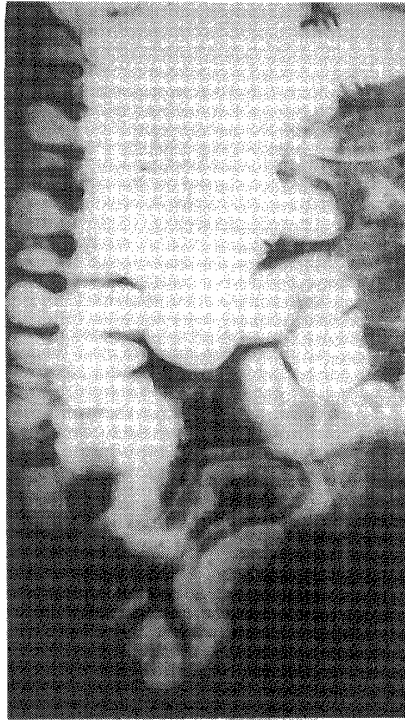


Figure 1 *T. saginata* (case 4) is clearly seen as a radiolucent shadows in the ileo-coecal segment.



Figure 2 *D. latum* (case 5) is observed in the ileo-coecal segment.



Figure 3 *T. saginata* (case 4) recognized as a long rediolucent shadow in the rectum before defecation.

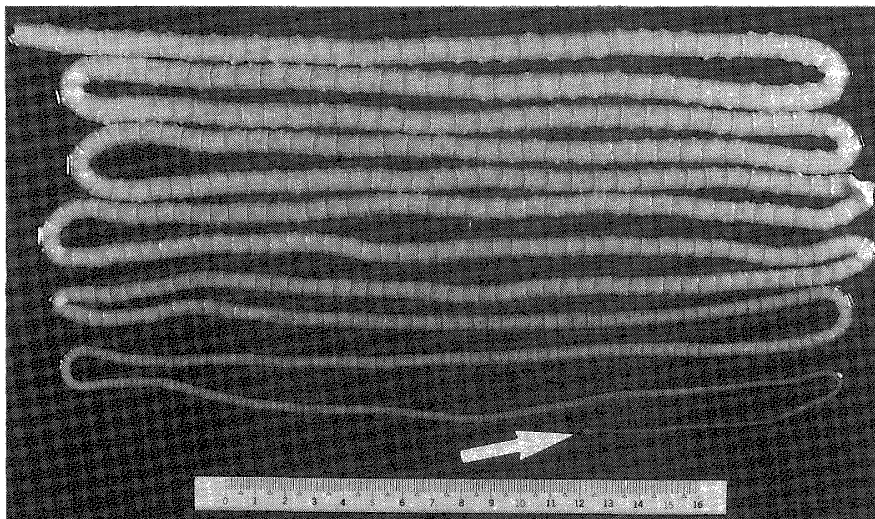


Figure 4 *T. saginata* (case 9) as the whole shapo with scolex (arrow). This worm was 275 cm in length.

In this study, we modified the treatment regimen and inserted dose of Gastrografin. Pretreatment in this method is based on the pretreatment of barium enema procedure. This procedure has an advantage that the feces in the intestinal lumen of the patients were less, and the initial dose of Gastrografin could be increased up to 300 ml from 100 ml used by Waki *et al.* (1986). According to this modified method, this drug is more effectively utilized.

Relatively new drugs such as paromomycin sulfate, niclosamide and praziquantel show a good curative efficacy (80~100%) in intestinal tapeworm infection (Salem and El-Allaf, 1969; Kihara *et al.*, 1973; Paz, 1977). However, in almost all cases, tapeworms were destroyed with these drugs and scolexes of expelled tapeworms were not found in their discharged feces.

In this method, all cases who have confirmed the tapeworm as a radiolucent shadows in the intestinal lumen were successfully treated with Gastrografin. Case 10 and 11 had discharged the long fragment of tapeworm before the first treatment, so that these tapeworms were too small to be recognised by X-ray monitor at the first treatment.

As regards the time required for this treatment (from 15 to 30 min) it was shorter than that of other medical treatment.

It is concluded that the present method is more effective for treating these intestinal tapeworm infection than other vermifuged methods. Therefore, we recommended that Gastrografin should be a drug of first choice for the treatment of intestinal tapeworm.

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ガストログラフィンによる広節裂頭条虫,
無鉤条虫, 大複殖門条虫の駆虫効果

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ガストログラフィンを用いて, 広節裂頭条虫症 (8例), 無鉤条虫症 (2例), 大複殖門条虫症 (1例) の条虫症, 計11症例に駆虫を試み良好な駆虫効果が得られた。駆虫前日に前処置として, 消化管注腸造影の前処置を応用し, 腸管内の食物残渣や糞便を除去し, 駆虫時のガストログラフィンと条虫体とが完全に十分接触できる状態にまで処理しておき, ガストログラフィンを十二指腸ゾンデでトライツ靱帯より300 ml から500 ml 注入し, 駆虫を行った。駆虫時, 条虫体が透視下にて, 透亮像として確認された症例は全例, 駆虫後頭節を確認することができ, 生きた状態で完全駆虫することができた。駆虫時間はわずか15分から30分で, 駆虫を完了することができた。

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ENDEMIC ONCHOCERCIASIS ON THE JARAWA VALLEY AREA OF PLATEAU STATE, NIGERIA

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Abstract: Clinical and parasitological survey on onchocerciasis was carried out in the Jarawa Valley area of Nigeria. A total of 1,821 inhabitants from six villages were examined and 908 (49.9%) were positive for microfilaria of *Onchocerca volvulus*. Onchocercal infection increased with age, reaching the highest level in adults over 40 years old. Pruritus was the most frequent lesion, followed by skin depigmentation, visual impairment, nodule, hanging groin and elephantiasis. Leopard skin occurred in various parts of the body including areas not exposed to blackfly-bites. Lymphatic manifestation, hanging groin and elephantiasis, occurred mostly in old male subjects. The adult had the highest prevalence of nodules mainly in the pelvic regions. Skin biopsies taken from the iliac crest gave the highest frequency of positive skin snip.

INTRODUCTION

Recently, Dipeolu and Gemade (1983) reported that about 750,000 Nigerians are suffering from onchocerciasis, with between 30,000 and 40,000 suffering from various ocular complications of onchocercal etiology. As a result of the serious socioeconomic liabilities caused to endemic communities of onchocerciasis in this country, the Federal Ministry of Health recently inaugurated National (Nigeria) Onchocerciasis Control Programme (NOCP). By this programme, the country was divided into zones for research convenience.

Few investigations were carried out on the Jos Plateau (NOCP Zone II) except by Roberts and Irving-Bell (1985), Onwuliri *et al.* (1987) and Nwoke *et al.* (1986) who showed that this disease and its vector species are widespread in this region. Many endemic communities in this zone are yet unidentified and unstudied. We report here results of clinical and parasitological surveys on the Jarawa Valley area of Nigeria.

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MATERIALS AND METHODS

Study area: The study area is the Jarawa Valley area in Central Nigeria, located in the northern part of the Jos Plateau. Six villages covered in this study. The six villages are located at different distance from the Jarawa river system, the main source of domestic water supply especially during the dry season. One of the main physical features of this area is the range of hills (Jarawa Hills) surrounding these villages. These highlands are the sources of a net-work of rivers and streams; most of which are breeding foci of *Simulium damnosum* (Nwoke, 1986). This area is in the northern Guinea savanna with yearly temperature between 12°C and 32°C.

The study area is inhabited mainly by rural farmers. Fishing, hunting and forest guards are exclusively performed by men. Because of the fact that this area has a special advantage of being tsetse-free together with a higher rainfall than the surrounding plains, animal husbandry is another major occupation of the nomadic Fulani.

Between January, 1985 and April, 1986, villagers were examined according to the onchocerciasis survey form (WHO, 1966). Mobile parasitology survey teams made up of the authors and their assistants collected skin biopsies and interviewed to ascertain name, estimated age, sex, occupation and village distance from breeding sites of vectors. The volunteers were physically examined for clinical manifestations of onchocerciasis. Visual acuity and blindness were evaluated as the inability to quickly count fingers at 3 m distance. Using a Holth-type corneo-scleral punch (2 mm), six bloodless skin snips (2 from shoulders, 2 from 5 cm above the lateral iliac crests and 2 from the calves) were collected. The microfilariae emerged in physiological saline of the wells of microtiter plate were counted in situ under a dissected microscope at 24 hr after the collection.

RESULTS

Microfilariae of *Onchocerca volvulus* emerged from one or more of the skin snips taken from 908 (49.9%) out of 1,821 subjects examined (Table 1). There was a gradual increase in the microfilarial rate with age with the highest infection rate of 72.1% recorded among subjects 40 years and above of age while the least (7.3%) was observed among 0-9 years of age.

Table 1 Microfilarial rate in villages by age

Village	Microfilarial rate in per cent by age in years					Total
	0-9	10-19	20-29	30-39	40+	
Maijemu	0.0 (0/56)*	24.0 (24/100)	65.0 (26/40)	72.2 (52/72)	75.0 (84/112)	48.9(186/380)
Dass	—	—	50.0 (8/16)	66.7 (16/24)	60.0 (72/120)	60.0 (96/160)
Rando	11.4 (4/32)	63.6 (28/44)	68.8 (44/64)	84.6 (44/52)	66.7 (48/72)	63.6(168/264)
Gwodong	16.2(18/111)	50.0 (45/90)	90.0 (54/60)	77.8 (21/27)	91.7 (99/108)	59.9(237/396)
Dorong	2.1 (3/141)	16.3 (24/147)	56.3 (27/48)	80.0 (24/30)	86.2 (75/87)	33.8(153/453)
Federe	0.0 (0/2)	60.0 (6/10)	16.7 (2/12)	52.2 (12/52)	40.5 (68/168)	52.2 (48/92)
Total	7.3(25/342)	32.5(127/391)	67.1(161/240)	65.8(169/257)	72.1(426/591)	49.9(908/1,821)

* (No. positive/No. examined)

Table 2 Prevalence of onchocercal lesions by villages

Village	Number examined	Number with onchocercal lesions:				
		Nodule	Visual impairment	De-pigmentation	Pruritus	Hanging groin & elephantiasis
Maijemu	380	10	14	26	38	10
Dass	160	8	44	20	48	4
Rando	264	28	16	28	60	0
Gwodong	396	33	21	63	75	0
Dorong	453	3	12	21	21	0
Federe	168	2	10	22	20	0
Total	1,821	84	117	180	262	14
(%)	(100.0)	(4.6)	(6.4)	(9.9)	(14.4)	(0.8)

Table 3 Prevalence of onchocercal lesions by age and sex

Sex	Age group (years)	Number examined	Nodule	Visual impairment	De-pigmentation	Pruritus	Hanging groin & elephantiasis
Male	0-9	183	2	0	0	5	0
	10-19	201	8	1	4	11	0
	20-29	131	6	13	18	20	2
	30-39	135	18	24	30	44	8
	40+	309	15	33	49	60	4
	Total	959	49	71	101	140	14
Female	0-9	159	0	0	0	2	-
	10-19	190	2	1	0	11	-
	20-29	109	11	8	13	28	-
	30-39	122	9	16	18	35	-
	40+	282	13	21	48	46	-
	Total	862	35	46	79	122	-

Table 4 Frequency distribution of onchocercal nodule by age

Age group	0-9	10-19	20-29	30-39	40+	Total (%)
Number examined	342	391	240	257	591	1,821
Number of nodule	5	16	18	24	31	94(100.0)
Anatomical region of nodule						
Head	0	1	1	0	2	4 (4.3)
Thorax	2	3	3	6	5	19 (20.2)
Upper limbs	0	1	1	4	8	14 (14.9)
Lumbar & abdomen	0	0	3	1	2	6 (6.4)
Pelvic region	3	11	9	8	12	43 (45.7)
Lower limbs	0	0	1	5	2	8 (8.5)

Table 5 Frequency of positive skin biopsy from different anatomical regions of the body

Anatomical region	Frequency of positive snip (%)
Scapular region	516 (56.8)
Iliac crest	798 (87.9)
Calf region	610 (67.2)
Total	908 (100.0)

The results showed that in four (Maijemu, Dass, Rondo and Gwodong) out of the six villages examined, there was no difference in the infection rate by sex. However males subjects showed a higher microfilarial rate than their females at Dorong and Federe, 40.7% in male and 29.3% in female at Dorong; 50.0% in male and 33.3% in female at Federe.

The result showed that the frequency of onchocercal lesions was different by villages. The least number of lesion was observed at Dorong with the least microfilarial rate (Table 2).

The most frequent lesion was pruritus (14.4%) and this was followed by skin depigmentation (9.9%), especially on the shin. The least occurring lesion was hanging groin and elephantiasis (0.8%) whilst nodule and visual impairment scored 4.6% and 6.4%, respectively.

Like in the microfilarial rate, the percentage clinical manifestations increased with age. Visual impairment and depigmentation were not observed in 0-9 years age group. Hanging groin and elephantiasis occurred only in older male subjects (Table 3).

A total of 94 onchocercal nodules were observed in the 84 nodule-positive persons (Table 4). Nodules were found most abundantly in the pelvic region followed by the thoracic region. The prevalence of onchocercal nodules varied with age, with lower rate occurring in the age group below 19 years old. Of the 25 persons of positive for microfilariae of *O. volvulus* in the age group 0-9 years, only five nodules were observed in two. Nodules occurred in all the regions of the body in the group 40 years old or more, with the highest prevalence in the pelvic region.

Of the 908 persons positive for microfilariae of *O. volvulus* the highest frequency of positivity of 798 (87.9%) occurred in the iliac crest, the scapular region and calf showed 56.8% and 67.2%, respectively (Table 5).

DISCUSSION

The present study demonstrates that 49.9% of the inhabitants were positive for microfilariae of *O. volvulus* in the Jarawa Valley, Nigeria. Thus, adapting the recommendation of WHO (1966), this area must be regarded as mesoendemic area for onchocerciasis. However, three (Dass, 60.0%; Rando, 63.6%; Gwodong, 59.9%), which were shorter distance from breeding sites than the other villages, out of the six villages were almost approaching hyperendemic situation. The microfilarial rate in the present study is much higher than 11.1% infection around the Assob river area (Onwuliri *et al.*, 1987) of the Zone II of the NOCP area. This comparatively high infection rate in the Jarawa Valley area may stem from the fact that these villages in the Jarawa District area are surrounded by the fast flowing tributaries of the Jarawa river system so that they have more breeding foci of vectors than

the other areas in the state (Nwoke, 1986).

The present study showed that there was a gradual increase in the microfilarial rate with age, similar to the findings of Enarson (1977) in Sudan and Dipeolu and Gemade (1983) in Nigeria. This situation bears witness to the chronic and cryptic nature of onchocerciasis infection. The microfilarial rate of 7.3% in children (0-9 years old) in the present study is attributed to early contact with infective blackfly bites in high endemicity. It is not clear the reason of a difference in prevalence by sex at two out of six villages in the present study.

Only 84 (4.6%) of the inhabitants examined had onchocercal nodules in the present study. This value is certainly low compared to nodules in other foci. In both sexes and ages, the great majority of the nodules occurred in the pelvic region followed by thoracic region and the head, similar to the results reported by Anderson *et al.* (1974) and Enarson (1977) in other African foci.

Visual impairment in the present study was found to be concentrated in the working age groups causing a lot socioeconomic liabilities unable to maintain the productive activity for long. The visual impairment rate in the present study was, however, comparatively low; 6.4% of the inhabitants examined (both impaired vision and total blindness). This is in contrast to 16.2% of the inhabitants (Budden, 1957), 10.3% (Dipeolu and Gemade, 1983) in other Savanna areas. This fact may be attributed to the habitual migration of disabled people in this part of the country to urban centers to beg for alms, consequently reducing the actual number of blind people in the rural communities. It may also be that the microfilariae of *O. volvulus* in the Jos Plateau has less toxicity than that of other Savanna region.

Leopard skin was observed in 9.9% of the villagers especially among the older age groups. It was frequently observed on the thigh, buttocks and the shin. The occurrence of leopard skin on sites not readily exposed to blackfly bites in the present study supports the fact that this striking manifestation is not caused only by dermatotoxic components of blackfly saliva as suggested by Craig (1932) and Nwokolo (1950). The present findings agree with the observation of Gibson *et al.* (1980), Connor and Palmieri (1985). On the contrary, the absence of leopard skin among younger age groups (0-19 years) supports the fact that this lesion is invariably associated with long-standing onchocercal infection.

All the cases of hanging groin and scrotal elephantiasis were observed among men of older age group. No such cases as described by Cherry (1959), Connor and Palmieri (1985) were observed among women. The association between onchocerciasis and elephantiasis had been recorded by several other workers including Buck *et al.* (1971) in Chad and Wonde *et al.* (1973) in Southwest Ethiopia. Although some investigators (Oomen, 1969; Price, 1972; Heather and Price, 1972) noted that elephantiasis could not be caused by onchocerciasis, a more recent study by Gibson and Connor (1978) noted that in Africans, antigens released from the microfilariae of *O. volvulus* lead to the deposition of immune complex in the tissues, which in turn causes inflammation and fibrosis and, eventually obstructive lymphadenitis. This process may cause hanging groin and, possibly, also elephantiasis.

There was a marked difference in the frequency of positive skin snip from different anatomical parts of the body. Of the three anatomical parts compared, iliac crest revealed the highest positivity snippings. This finding corresponds to a finding that the greater number of onchocercal nodules observed around the pelvic region and support the idea of Kershaw *et al.* (1954) who found that microfilarial distribution in the skin is closely related not only to clinical manifestation, but also to the location of onchocercomata.

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ナイジェリア，プラトー州ジャラワ溪谷における
オンコセルカ症の流行状況

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ナイジェリア，プラトー州ジャラワ溪谷においてオンコセルカ症の臨床的，寄生虫学的調査を1985年1月から1986年4月に実施した。6集落1,821名の住民を検皮し，908名（49.9%）の住民が *Onchocerca volvulus* ミクロフィラリア陽性であった。オンコセルカ症の感染率は加齢とともに増加し，40歳以上の年齢群で最も高率であった。オンコセルカ症の病変は，掻痒疹が最も高率で，次いで皮膚の脱色素斑，視力障害，皮下腫瘤，鼠径下垂・象皮病の順であった。皮膚の脱色素斑 leopard skin は下肢だけでなく，臀部等のブユの吸血に直接さらされない部位にも認められた。鼠径下垂・象皮病のリンパ系の病変は，主に高齢の男性に認められた。また，皮下腫瘤は成人に多く，主に腰部に認められた。皮膚の生検では，腸骨部で最もミクロフィラリアの陽性率が高かった。

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