

Malaria Vectors and Approaches to their Control

**in malaria affected countries of
the WHO European Region**

The proceedings of a Regional Meeting
on Vector Biology and Control

Almaty, Kazakhstan
3–5 May 2001

ABSTRACT

The purpose of the meeting was to discuss *Anopheles* spp., their role as malaria vectors, and their distribution in various eco-epidemiological zones of the WHO European Region. Also discussed were the susceptibility of mosquitos to insecticides and technologies for vector control, including biological methods. Entomologists and geneticists contributed knowledge on issues such as taxonomy, biology, ecology and the genetics of malaria vectors. The meeting recommended that WHO continue to support the expansion of the knowledge base and skills in medical entomology and support entomological research, including the resistance of malaria vectors to insecticides. The countries represented at the meeting were Armenia, Azerbaijan, Georgia, Israel, Kazakhstan, Kyrgyzstan, the Russian Federation, Tajikistan, Turkey, Turkmenistan and Uzbekistan.

Keywords

MALARIA – prevention and control
MOSQUITO CONTROL – methods
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1. Introduction

A Regional Meeting on Vector Biology and Control was held 3-5 May 2001 in Almaty, Kazakhstan. Participants included country representatives from national malaria control programmes (Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Russian Federation, Tajikistan, Turkey, Turkmenistan and Uzbekistan), research institutions (Israel, Kazakhstan, Russian Federation and Turkmenistan), the private sector (Kazakhstan), WHO staff members (EURO and Kazakhstan) and observers from the Centre of Biological Control in Israel.

The meeting was convened (1) to review the current status of knowledge in the area of vector biology and control, (2) to identify *Anopheles* species of malaria vectors and their role and distribution in different eco – epidemiological zones of the WHO European Region, (3) to review and discuss available information on mosquito susceptibility to insecticides in the WHO European Region, and (4) to review existing methods and technologies for vector control and their applicability, taking into account different eco – epidemiological settings, economic considerations and environmental concerns in the WHO European Region.

The meeting proceedings included a review of the current malaria situation, its recent history and progress with roll back malaria in malaria-affected countries of the WHO European Region, and an in-depth discussion of the status of mosquito vector resistance to insecticides, and the issues of biology, taxonomy, ecology and genetics of malaria vectors in malaria-affected countries of the WHO European Region. Many of the most modern approaches and techniques available for the determination of the physiological age of mosquitoes and various options for vector control, in particular adult mosquito control by means of residual sprays with insecticides, biological control, insect growth regulators, larvivorous fish, genetic control methods, and impregnated mosquito nets were discussed. The issues of resistance and irritability of malaria vectors to insecticides were also thoroughly considered.

Reports by well-known experts in the area of medical entomology and genetics contributed to the expansion of the participants' outlooks on issues concerning the taxonomy, biology, ecology and genetics of malaria vectors, as well as on the role and significance of vector control interventions in the European Region.

It was recommended for WHO (1) to continue assisting national malaria control programmes in planning, implementation and evaluation of vector control operations, and capacity building aimed at improving knowledge and developing skills/competence in the field of medical entomology, and (2) to continue to support financially and technically the carrying out of operational entomological research including monitoring resistance/irritability of local malaria vectors to insecticides used in both the agricultural sector and vector control.

It was recommended for WHO and Member countries (1) to continue studying the taxonomy of local *Anopheles* species and the areas of their distribution, (2) to continue studying the morphological differences of members of *An. maculipennis* complex, (3) to continue studying the life cycle of local *Anopheles* mosquitoes and their ecology, in particular the frequency of repeated feedings on man during a gonotrophic cycle,

and anthropophilism of local *Anopheles* mosquitos and ecological preferences of local *Anopheles* larvae, (4) to continue regular monitoring resistance/irritability of local malaria vectors to insecticides used in vector control and the agricultural sector, (5) to carry out study trials in order to determine the efficacy of larvivorous fish in different eco – epidemiological settings of European Region, (6) to continue carrying out trial studies on the efficacy of different biological control methods in reducing malaria transmission, and (7) to continue studies on genetic control techniques including the use of insect growth regulators.

It was recommended for Member countries (1) to continue indoor insecticide spraying as a principal vector control option (*against endophilic species of Anopheles mosquitoes*) in order to control epidemics/outbreaks of malaria in the WHO European Region, (2) to base decisions on the use of insecticides for vector control upon entomological and epidemiological assessments of local malaria situations, (3) to rotate various insecticides in order to prevent the development of irritability in malaria vectors, and (4) to initiate larvivorous fish rearing and distribution campaigns (*in areas under influence of exophilic or semi-exophilic species of Anopheles mosquitoes*) in combination with *Bti* and/or impregnated mosquito nets, if required.

It was recommended for WHO and RBM partners and donors (1) to continue to assist malaria-affected countries in the procurement and delivery of insecticides and equipment/supplies for vector control, with priority given those countries which are facing ongoing epidemics/outbreaks.

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3. Review

3.1 The malaria situation and progress with Roll Back Malaria in affected countries of the WHO European Region

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By the beginning of the 1960's, malaria had been nearly eradicated in all countries of the European Region, with the exception of some districts in Azerbaijan, Tajikistan and Turkey, where malaria transmission remained in residual foci.

Azerbaijan

As a result of a decrease in the attention placed on epidemiological malaria surveillance and the expansion of agricultural irrigation, some old foci of malaria once again became active in the Shirvan area of the Kura-Araksin lowland of Azerbaijan. By the end of the sixties, *An. sacharovi*, the principal vector of malaria, had developed resistance to DDT and other groups of insecticides extensively deployed in the agricultural sector and for malaria control. This led to a considerable degradation of malaria control activities, and the above-mentioned factors combined brought on an outbreak of *Plasmodium vivax*. By 1970, 23 districts and 4 towns of the republic had been affected. A series of heavy, complex malaria control interventions, with particular emphasis on mass inter-seasonal chemoprophylaxis with primaquine and indoor residual spraying with DDT, made containment of the outbreak possible, and in the following years, a gradual reduction in the incidence of *Plasmodium vivax* and the number of active foci of malaria was observed.

In 1978, 42 active foci (as compared to 149 in 1974) were registered in the republic, with only two amongst them reporting more than six malaria cases. Despite a significant reduction in malaria incidence, transmission in the republic was not interrupted. Owing to intense population migration, the residual foci have accounted for aggravation of the malaria situation and, since 1979, the republic has faced a new rise in its incidence. In 1984, 744 malaria cases were registered. Taking into account the serious health threat presented by the unfolding situation, the Ministry of Health of Azerbaijan undertook the measures necessary to prevent a large-scale malaria epidemic outbreak. While during the first stage (1981-1982), all efforts were focused on containing ongoing large-scale outbreaks, the second stage of activities (1983-1985) were aimed towards elimination of remaining foci and the building up of capacities for the complete eradication of malaria. In contrast to preceding years, particular attention was paid to disease management activities, and large-scale seasonal chemoprophylaxis was given topmost priority. This was connected with the fact that amongst the population in endemic areas, high rates of G6PD deficiency (15-30%) were observed. Additionally, the entire population within all affected areas was covered by malaria control activities. These efforts by and large succeeded in bringing about a substantial reduction in malaria incidence, and reduced rates were observed in all malaria-affected districts in the republic. Regardless of the reinforcement of malaria control interventions, the malaria situation in the republic remained uncertain. Although

outbreaks were contained, the complete interruption of malaria transmission in the republic was not achieved.

In the years to follow, sporadic cases were reported on an annual basis. The political and socio-economic consequences of the breakup of the USSR in the early nineties weakened malaria control interventions and led to the complete cessation of hydro-engineering and melioration. Population migration as well significantly complicated the malaria situation in Azerbaijan, resulting in a drastic rise of malaria morbidity. In 1996, the number of malaria cases reached 13,135, with the majority of these cases registered in the districts of Kura-Araksin and Lenkoran lowlands, areas which had been considered highly malaria-endemic in the past. In 1997, the situation was aggravated as a result of mudslides throughout the Kura-Araksin and Lenkoran lowlands, when mosquito breeding sites increased dramatically. The highest rates of malaria morbidity were reported in several districts of Azerbaijan bordering Iran, Georgia and Russian Federation.

With substantial financial and technical assistance from international institutions such as World Health Organization, Red Cross and Crescent International Federation, UNICEF, UNDP, World Bank, "Medicins Sans Frontiers" and the Italian oil company "Eni," the Ministry of Health of Azerbaijan initiated malaria control interventions within the context of the Regional "Roll Back Malaria" Programme. By 1997, the continuing increase in malaria morbidity was curbed. Since 1999, indoor residual spraying with synthetic pyrethroids has been resumed in most affected settlements. Over the course of the four years between 1997-2000, the malaria situation in the country continued to gradually improve, with only 1,526 cases registered in 2000.

There are both principal and secondary malaria vectors in Azerbaijan: *An. maculipennis* (the area of the Big and Small Caucasus), *An. sacharovi* (Kura-Araksin and Lenkoran lowlands), and *An. subalpinus* (Lenkoran lowland). In four pilot districts of the country, nurseries for the rearing of gambusia fish for larval control have been constructed in order to distribute the fish to other potentially risky areas of the country. In 2001, WHO funded operational studies on the efficacy of eucalyptus planting as a means of bringing about a reduction in breeding sites of malaria vectors, and the planting of these trees was subsequently initiated in one of the country's districts.

The diagnosis and treatment of malaria cases are carried out mostly on an outpatient basis, and the majority of cases are determined by active case detection (70% in 2000). With financial and technical assistance from WHO, drug efficacy monitoring trials were initiated in mid-2001.

Tajikistan

Malaria was practically eradicated in Tajikistan by the end of the sixties. In 1966, just 11 malaria patients were reported. Following 1961, preventive activities were undertaken within epidemic-prone areas, whereas in areas of residual endemicity (the Pyanj river basin), a complex of interventions including indoor residual spraying with DDT, active case detection and radical treatment of malaria cases was carried out. The importation of malaria by infected mosquitoes from bordering territories was presumed one of the main reasons of the area's residual endemicity. Many publications were dedicated to issues on the flying range and water-barrier crossing abilities of mosquitoes. By the end of the 1970's - beginning of the 1980's, it had been established

that malaria vectors (*An. pulcherimus* and *An. hyrcanus*) in southern Uzbekistan were easily capable of flying over the Amu-Darya River and back, a river over 750 meters in width. This provided solid evidence of the water-barrier crossing abilities of local infected species.

From 1963-1980, 135 malaria cases were detected in 25 settlements in seven of eight districts bordering Afghanistan (with the exception of the Kumsanghir district). Almost all these settlements were situated within 3 km of the floodplain areas of the Amu-Darya and Pyanj rivers, with Afghan settlements located just on the opposite side. Risk areas as far as incoming infected mosquitoes were concerned included Pyanj and all of the eastern part of its district, where many breeding sites existed along both sides of the border, and intense foci on the Afghan side. Of particularly high risk was the status of the western part of Parhar district, due to its many paddies and high vector densities. Within the Moscow district, the majority of high-risk areas included the mountainous stretches along the border, where mosquito crossings over a particularly narrow part of the Pyanj river were highly probable, and some persistent foci were located on the Afghan side. The situations in Lenin and GBAO districts were not termed high risk, as vector densities here were comparatively low along both sides of the border, and there were no malaria patients in the adjacent areas. The absence of malaria in the Kumsanghir district gave further proof of the hypothesis of malaria importation by infected mosquitoes from Afghanistan, as Afghan settlements across the border were more than 10 km away from the Pyanj river. On the other hand, there was little doubt about the presence of local transmission, which was not effectively contained owing to the exophilic behaviour of *An. pulcherimus* and *An. hyrcanus* vectors, as well as to a significant drop in the quality of epidemiological malaria surveillance. The latter was confirmed by local malaria outbreaks - 34 cases in Kalai-Humb district in 1971, and 90 cases in Kuliab in 1978.

From 1979 to 1981, the number of malaria cases rose from 58 to 121. Almost all cases were detected in the same districts in which sporadic cases had been routinely registered and local outbreaks had taken place from 1963 to 1980 (Pyanj, Moscow, Parhar, Kuliab, Vosei districts and the town of Kuliab). Most probably, two factors were responsible for the dramatic increase in morbidity in 1981. Before 1979, all the outdoor areas with districts engaged in cotton growing had been heavily sprayed with DDT, an action that undoubtedly curbed the growth of malaria vector densities, particularly on the part of the exophilic mosquito population. In 1979, the outdoor spraying of DDT for agricultural purposes was discontinued. On the other hand, all malaria control interventions carried out by the Soviet antimalarial teams in areas bordering Afghanistan were terminated as well, leading to a drastic deterioration of the malaria situation. A peculiarity of the epidemic process in 1981 was the rapid spread of infection to 54 settlements, accompanied by comparatively low transmission rates. The greatest number of cases (105) was reported in the Pyanj district. The morbidity rise in 1982-1983 also took its toll on Parhar and Moscow districts. The course of the malaria situation in Tajikistan 1981-1983 vividly demonstrated the fact that the system of malaria control interventions carried out at that point in time was ineffective both in terms of prevention of the occurrence of new foci and the containment of the intensity of transmission in existing foci. In the absence of effective residual insecticides, treatment and preventive activities constituted the major part of complex efforts to sustain malaria transmission during these years. The use of malathion as an alternative insecticide for indoor spraying and mass seasonal chemoprophylaxis, in hand with upgrading the level of disease management and preventive interventions, did result in

a reduction of malaria incidence in the republic in the years to follow. Despite large-scale anti-malarial activities, however, malaria cases remained within a range of 200-300 per year.

The malaria situation started to deteriorate from the beginning of the 1990's. As significant numbers of refugees returned to their country from Afghanistan, the mass importation of malaria to formerly malaria-free districts, in particular, to the Kurgan-Tjubin area, was observed. During the civil war, the country's health care system was brought to a halt and all malaria control interventions were discontinued. In these years (1994-1995), just 2,410 and 6,103 malaria cases were reported respectively, with official statistics evidently failing to illustrate the real malaria situation in the country. In 1997, 29,794 malaria patients were registered, although, according to a WHO expert assessment, this figure was presumably higher than 200,000. From 1997 to 1999, due to the implementation of large-scale malaria control interventions, with special emphasis placed on vector control activities (indoor residual spraying with insecticides), the officially reported morbidity decreased by more than 50%, with numbers falling from 29,794 to 13,493. However, the malaria situation has remained very complicated, manifested in the first place by the further spread of the disease along the country's territories and, secondly, by the re-establishment of local transmission and a growing number of *Plasmodium falciparum* cases. An increase in the number of malaria cases during 1999-2000 was observed, with the main reasons for this, presumably, the decrease in the volume of indoor spraying and all-round improvements in both case detection and reporting in 2000.

The significant financial and practical assistance on the part of such international bodies as WHO, UNICEF, ECHO, World Food Programme, United States Agency for International Development (USAID), MERLIN, ACTED and the national governments of Japan, Norway and Italy has played a fundamental part in the containment of large-scale epidemics in the country. However, despite evident improvements in the system and delivery of malaria control interventions over the last years, the range of malaria-related problems and their possible resolution is yet to be defined, since existing statistical data cannot provide a full and comprehensive picture of malaria distribution in the country. At present, WHO, in collaboration with the Ministry of Health of Tajikistan, has initiated operational research aimed at estimating the true extent of the current malaria problem in the country.

Malaria vectors in Tajikistan include *An. superpictus*, *An. pulcherimus* and, possibly, *An. hyrcanus*. The results of studies on vector resistance to insecticides (DDT, fenitrothion, cyfluthrin and deltamethrin) have revealed that all vectors were susceptible to the above-mentioned insecticides. From 1998-2000, an ever-increasing number of malaria cases has been observed in the areas of Tajikistan bordering with Uzbekistan and Kyrgyzstan.

In June 2001, the World Health Organization, in collaboration with Ministries of Health of Tajikistan, Uzbekistan and Kyrgyzstan, initiated and conducted a Malaria Border Coordination Meeting aimed at outlining a direction and strategy for the increased coordination of malaria control in border areas of these countries. The country representatives concurred that all necessary steps should be taken to improve coordination among the participating countries for solving common problems in the control and prevention of malaria.

Turkey

Since 1960, as a result of a large-scale malaria control programme initiated in the country, malaria morbidity rates were reduced and endemic areas decreased on an annual basis. Within the period between 1960 to 1974, just once, in 1964, did the number of malaria cases exceed five thousand. In the mid 1970's, following the country's success in the field of malaria control, the national malaria control program was re-organized, and both malaria control and surveillance fell into the hands of primary health care system personnel. This coincided with the commencement of the construction of dams and water power plants on the Chukurova plain, which led to the migration of workers from eastern malaria-endemic districts of the country. Massive malaria importation, along with irrigation and the growth of malaria vector populations, resulted in the outbreak of severe *Plasmodium vivax* epidemics in the Adana, Icel and Hatay provinces in 1974. In 1977, 115,512 malaria cases were registered in the country. Owing to large-scale malaria control interventions including disease management activities, vector control interventions (indoor residual spraying and larvicidal activities based on the use of chemical insecticides and larvivorous fish) and, finally, intensified malaria surveillance, the epidemic was contained in 1978.

In the beginning of the 1980's, the Chukurova plain remained the most malaria-affected area of Turkey. This was caused by conditions favorable for malaria transmission; difficulties in conducting interventions for vector control due to the exophily of some local vectors; socio-behavioral attitudes on the part of local populations which had allowed for extended man-vector contact; and the mass influx of citizens from malaria endemic districts, particularly the south-eastern areas of Anatolia. Moreover, the heavy use of insecticides in the agricultural sector led to the development of *An. sacharovi* resistance to DDT (1959) and dieldrin (1971). The attitude on behalf of the population towards residual spraying with malathion was negative, although, in those years, it was still an effective insecticide. All of the above-mentioned factors brought about a new rise in malaria morbidity in 1980-1986, affecting Adana, Osmani, Sanljurfa, Mardin, Adiaman and Icel provinces. In 1983, about 70,000 cases were reported in the country. The application of a new residual insecticide, pirimiphos-methyl, along with the intensification of malaria surveillance, allowed for a significant reduction of malaria cases in 1984-1990. In 1990, the lowest malaria morbidity level to be seen over the past 15 years was reported in the country.

The success which had been achieved, however, was not for long, and from 1991, a gradual rise in malaria incidence has been witnessed. In 1993-1994, a large-scale epidemic spread to GAP, a development project in south-east Anatolia, particularly within the Diarbakir, Batman, Seert, Sirnak and Mus provinces. In 1994, according to statistical data, 84,345 malaria cases were reported, although evaluation indices were pointing to a much higher number (from 160,000 to 170,000). Some of the large-scale control interventions carried out included indoor spraying with insecticides, intensified surveillance on malaria, use of larvicides and growth regulators, Bti-based agents, larvivorous fish, and ultra-low-capacity sprayings with pyrethroids. Special attention was given to health education amongst the population.

Throughout 1994-2000, the malaria situation in the country steadily improved. Despite the fact that only 11,432 malaria cases were reported in 2000, the problem remained urgent. The ongoing reduction in malaria morbidity over the past years was most probably not only the result of malaria control interventions but also a consequence of

changes in climatic and environmental conditions (low rainfall over the past two years) which adversely affected malarial mosquito survival and their capacity to transmit malaria. To date, more than 15 million people (23% of the total population of Turkey) continue to reside in the country's malaria endemic districts, and 44% percent of the population lives in areas at high risk to the resurgence of malaria transmission. Thus, despite the significant decrease of malaria morbidity over the past years, the malaria settings, as we know by experience, may be subject to sudden (and negative) changes. In light of the current situation, it is vitally important to consider the intensification of epidemiological surveillance of malaria at provincial levels, especially in southeastern Anatolia, where the malaria situation remains serious.

At present, due to a financial and socio-economic crisis within the country and limited governmental support to health care programs (including units involved in malaria control), the value of the assistance on the part of international institutions cannot be underestimated. The financial assistance provided (or to be provided) to the national level "Roll Back Malaria" program by international bodies such as WHO, UNDP, Administration of State Project Development, UNICEF and the World Bank will undoubtedly prove of great help to the Ministry of Health of Turkey in the area of malaria control and the prevention of epidemics.

Armenia

By 1963, malaria had been entirely eradicated in Armenia. The situation remained stable until 1994, when approximately 196 malaria cases were registered amongst military personnel. In subsequent years, a drastic reduction in malaria control intervention capacities and a weakening of the malaria surveillance system resulted in a steady increase in the number of malaria cases, reaching 1,156 by 1998. Over 98% of these cases were detected in the Masis district of the Ararat river valley, an area bordering Turkey. *An. maculipennis* serves as the main malaria vector in the country.

Owing to efforts and significant assistance on the part of WHO, UNICEF, UNDP, International Federation of Red Cross and Red Crescent Societies, World Food Program, World Bank and the governments of Italy and Norway, the spread of malaria epidemics has been interrupted.

Over the most recent years, the numbers of malaria cases have continued to decrease, dropping to 141 in 2000. In carrying out malaria control interventions, main emphasis is placed on indoor spraying in the most malaria-affected settlements, upgrading existing malaria diagnostic and treatment methods, mass preventive activities, improvement of epidemiological surveillance systems, health staff training, and health education amongst the population.

In addition to *An. Maculipennis*, other malaria vectors in Armenia include *An. sacharovi* and *An. claviger*. The appearance of *An. sacharovi* (the main malaria vector in Transcaucasia) in the Ararat valley districts has created conditions more favorable for malaria transmission in the country. All *An. maculipennis* populations that were tested for resistance to cyfluthrin were found susceptible to this insecticide.

The main target for Armenia in next two years will be not only the maintenance of the epidemiological well-being which has been attained, but also the elaboration of ways

and approaches for the further reduction of malaria morbidity, with the ultimate objective of the complete eradication of malaria within the country.

Georgia

By 1970, malaria had been completely eradicated in the country. From 1970 to 1995, only 139 imported malaria cases were registered. In the following years, owing to a drastic reduction in the activities aimed at prevention of malaria transmission, as well as the resurgence and intensification of migratory processes, the malaria situation in the country deteriorated considerably. The first three cases of local malaria transmission were detected in 1996 amongst the residential population within a district bordering Azerbaijan. In subsequent years (1996–2000), the number of malaria cases continued to increase, reaching 245 in 2000. The situation is growing still worse, with the first instances of local malaria transmission registered in western Georgia in 2001. The conditions favorable for local malaria transmission are present in almost 52% of the country's territory, an area which is home to some 93% of the population.

Taking into account the highly complicated malaria situation in the country in the past and the present day conditions favorable to a resurgence in local malaria transmission in the majority of the country's areas, as well as the steady increase in malaria case numbers, the WHO Regional Office for Europe, in collaboration with the Ministry of Health of Georgia, initiated and conducted a national meeting on the "Roll Back Malaria" partnership program in order to draw potential donor attention to the situation. In 2000, WHO increased the amount of financial and technical support provided to the national malaria control program. With the assistance of WHO over the past several years, national health personnel have undergone training both at home and abroad on the topic of malaria control; diagnostics and laboratory equipment, insecticides, and medicines for malaria treatment. A computer and car were also purchased with the assistance of WHO, and funding was provided to carry out epidemiological surveillance on malaria. WHO officials and experts visiting the country offer guidance and knowledge in the evaluation of the local malaria situation and may contribute to the decision-making process regarding issues on malaria control.

At present, the highest risk of the resurgence of malaria transmission and its spread concerns the areas bordering with Azerbaijan and Armenia in eastern Georgia, the Black Sea coastal areas, and Kolhid lowland districts in western Georgia, areas in which over 68% of the country's total population reside, with an epidemiological season of over 150 days. The main and secondary vectors there include *An. maculipennis*, *An. claviger*, *An. superpictus*, *An. sacharovi*, *An. hyrcanus*, and *An. melanoon*. A lower degree of risk of the resurgence of malaria transmission and distribution also concerns territories which are home to 18% of the total population, with an epidemiological season of 90 to 120 days. The vectors there include *An. maculipennis*, *An. claviger*, *An. superpictus*, and *An. plumbeus*.

Turkmenistan

Following large-scale malaria control interventions, malaria in Turkmenistan was entirely eradicated in 1960. From 1965-1980, 23 local malaria cases were registered in Maryi and Ahal veloyats, areas bordering other countries. Military personnel returning from service in Afghanistan brought in the majority of imported cases which were registered throughout the 1980's. The continuing deterioration of the epidemiological

situation in malaria was exacerbated by an outflow of well-trained health personnel and a drop in the quality of public health services. The attention paid towards epidemiological surveillance on active and potential foci diminished. A number of significant environmental changes in the last years have caused an increase in mosquito breeding sites. Construction of 17 large water storage facilities in the Karakum canal basin zone was followed by the formation of vast filtration reservoirs capable of serving as breeding sites for malaria vectors. There are seven malaria vectors in Turkmenistan, most dangerous among them *An. superpictus*, *An. pulcherimus* and *An. maculipennis*. The monitoring of *An. superpictus* susceptibility to cyfluthrin, ICON, DDT and propoxur in Lebap, Maryi, Ahal, Dashogus and Balkan veloyats has revealed that all the above insecticides remained highly effective for indoor spraying. The first local malaria cases were registered in 1996-1997. By 1998, the malaria situation had taken a drastic turn for the worse, and 108 malaria cases, or 78% of all cases reported in the country for that year, were detected within the Gushgin etrap of Maryi veloyat. The majority of these cases were observed in the training centre "Fakel." To prevent the further spread of malaria throughout the etrap area, personnel carried out seasonal chemoprophylaxis with chloroquine and indoor spraying. These interventions allowed for a significant decrease in malaria morbidity within the focus area. Presumably, local malaria transmission appeared as a result of a malarial pathogene imported by mosquitoes flying in from bordering Afghanistan. In 2000, 18 local malaria cases were reported in Turkmenistan.

Taking into account the high risk of the resurgence of malaria transmission and distribution in Turkmenistan, the World Health Organization, in collaboration with the Ministry of Health of Turkmenistan, carries out various measures including the training of health staff, the purchase of diagnostics and laboratory equipment, medicines, insecticides and corresponding equipment; intensification of epidemiological surveillance activities; expert assessment of local malaria situation and problems related to its prevention, as well as other steps necessary to prevent malaria outbreaks, maintain the epidemiological well-being of the country, and, finally, to reach the goal of the complete eradication of malaria.

Uzbekistan

Malaria was eradicated in Uzbekistan in 1961. Even within the post-eradication period, however, the task of malaria prevention within the republic retained its urgency, as many of the country's areas are highly malariogenic, the importation of malaria continued, and the risk of the resurgence of local malaria transmission remained. Throughout the 1980's, isolated instances of malaria cases were registered, with most cases imported by military personnel returning home following tours of duty in Afghanistan.

Taking into account the malaria situation in neighboring Tajikistan at the beginning of the 1990's and the high risk of resurgence of local transmission, particularly in areas bordering Tajikistan, the Ministry of Health of Uzbekistan initiated and conducted a number of activities aimed at the intensification of epidemiological surveillance on malaria. From the mid 1990's to date, the number of imported malaria cases has continued to increase (21 cases in 1994 and 80 cases in 2000). In 1999, due to a steady increase in imported malaria cases and the presence of conditions favorable for malaria transmission, the first local cases of malaria, seven in all, were registered. By 2000, the number of locally transmitted malaria cases had increased considerably,

reaching 46. All registered cases occurred in the Surhandaryin area bordering Tajikistan. In 2000, by order of the Ministry of Health of Uzbekistan, a national program on malaria surveillance was adopted. To reinforce malaria control interventions in three areas bordering Tajikistan, targeted malaria control services were created and made operational. In May 2001, a draft resolution entitled "On the intensification of activities aimed at malaria prevention and vector control in the Republic of Uzbekistan" was presented for consideration to the Cabinet of Ministers.

There are seven *Anopheles* species registered within the territory of Uzbekistan: *An. superpictus*, *An. maculipennis*, *An. pulcherimus*, *An. martinius*, *An. claviger*, *An. hyrcanus* and *An. algeriensis*. The monitoring of vector susceptibility to insecticides has revealed that only *An. superpictus* populations in Fergan were resistant to malathion, fenitrothion, bendiokarb and propoxur. All other vectors noted, including *An. maculipennis* and *An. pulcherimus*, remain susceptible to nearly all commonly used insecticides.

In view of the real hazard of malaria distribution along the country's territories, the World Health Organization provides significant financial and technical assistance to national health care services in the area of malaria prevention and control. Over the past several years, through the assistance of WHO, local health staff has undergone specialized training on malaria control both at home and abroad, and diagnostics and laboratory equipment, medicines for malaria treatment, insecticides, computers and cars have been purchased. Funding to cover the costs of carrying out epidemiological surveillance upon malaria has been provided. WHO has also contributed to health education activities aimed at improving knowledge on preventive measures amongst the population, as well as research on the resistance of malaria vectors to insecticides. WHO staff and experts visiting the country provide assistance and practical advice in the evaluation of the local malaria situation and prevention-related issues.

In June 2001, the World Health Organization, in collaboration with the Ministries of Health of Uzbekistan, Tajikistan and Kyrgyzstan, initiated and conducted a meeting on the coordination of malaria control activities within the border areas of these three countries. The representatives of participating countries agreed that all necessary measures should be taken to enhance coordination between the countries in order to resolve mutual problems related to malaria prevention and control in bordering areas.

Kazakhstan

The last local case of malaria was registered in 1967, and from 1968 on, only imported cases were reported in the country. However, the epidemiological situation grew more complicated by the beginning of the 1990's, when Kazakhstan acquired independence, and international contacts, including those with malaria endemic countries, expanded considerably. From 1990-1997, a growth in the number of imported malaria cases was registered in the country, and the first malaria case due to local transmission was reported in 1992. In 2000, 7 local malaria cases were registered within the area of South Kazakhstan and Almaty.

The ecological and climatic features within the majority of the country's area leads to a high risk towards the resurgence of local malaria transmission following its import. *An. messeae*, the most common malaria vector in the republic, is found throughout most of the country's territory. Studies on this vector's resistance to different insecticides has revealed that DDT-resistance was highest (up to 77%) in Uralsk (and nearly absent in Ust-

Kamenogorsk), and that resistance to malathion and fenitrothion was virtually absent in all the areas under study. Neither was resistance detected to synthetic pyrethroids - deltamethrin, cyfluthrin and Karate. The differences in eco-climatic settings, types of landscape, vector species, structure and occupational patterns of population and migratory levels define the heterogeneity in malariogenic potential of the country. The highest risk of the resumption of malaria transmission concerns Almaty (10 districts and two cities), Jambyl (5 districts), South-Kazakhstan (7 districts and 1 city), West-Kazakhstan (1 district and Uralsk), some areas of East-Kazakhstan (3 districts), and the cities of Almaty, Astana and Karaganda. The population of these regions at risk of malaria stands at over 4.5 million. In 1998 and 1999, the Ministry of Health of Kazakhstan put forward resolutions aimed at the intensification of epidemiological surveillance of malaria for all health care institutions of the country. The National Plan of Action on malaria prevention in 2001-2003, passed in 2000, is presently being carried out.

Taking into account the possibility of a further decline in the country's malaria situation, the World Health Organization provides assistance in staff training, the purchase of diagnostics and laboratory equipment and medicines for malaria treatment, the carrying out of scientific research, expert assessments of local malaria situations and problems/constraints faced.

Kyrgyzstan

In Kyrgyzstan, malaria was eradicated in 1959. However, from 1986 onwards, as a result of the importation of malaria by ex-service military upon return from Afghanistan, some local cases were registered. In 1986 and 1987, 14 and 10 cases respectively of local malaria were detected. In 1988, there were 21 cases due to local transmission, with 11 registered in the Batken district, which borders Tajikistan and Uzbekistan. In the years to follow (including 1995), only imported cases were reported. In 1996, after a long break in local transmission, the first case of autochthonous malaria was registered in the Panfilov district. From that point forward, despite the reduction in imported malaria, there has been a rise in the number of autochthonous malaria cases. In 2000, 7 malaria cases were reported.

The highest malariogenic potential belongs to the southern regions of the country, including the Osh, Batken and Jalal-Abad areas, primarily due to the many rice plantations located there. Malaria vectors in these areas include *An. pulcherimus*, *An. superpictus*, *An. hyrcanus*, *An. martinius*, *An. claviger* and *An. messeae*. Studies on vector resistance to different insecticides have revealed that all the above-named species are susceptible to DDT, fenitrothion, cyfluthrin, deltamethrin, malathion, lambda-cyhalothrine and propoxur.

Taking into account that the country's malaria situation could further deteriorate, the World Health Organization provides assistance in staff training, the purchase of diagnostics and laboratory equipment and drugs for malaria treatment, the carrying out of scientific research, expert assessment of local malaria situations and problems/constraints faced.

In 2000, almost all local malaria cases registered in the country were found in areas bordering Tajikistan. In June 2001, as a result of a worsening of the malaria situation in districts bordering Tajikistan, Uzbekistan and Kyrgyzstan, the World Health Organization, in collaboration with national Ministries of Health, initiated and carried out a meeting on

the coordination of malaria control activities in border areas of these three countries. The representatives of participating countries concurred that all necessary measures must be taken to improve coordination among the countries in order to resolve mutual problems related to malaria prevention and control in border areas.

Russian Federation

Throughout the 1980's, local malaria cases were reported in the Orenburg, Pavlodar, Tyumen, Chelyabinsk, Omsk, Tomsk, Gorky, and other regions of Russian Federation. This was due in large part to the continuous introduction of malaria from Afghanistan. From the beginning of the 1990's, the malaria status of the country was further -aggravated.

Throughout 1995 to 1998, the numbers of imported and local malaria cases continued to rise, with the peak reached in 1998, with 1,018 and 63 cases respectively. In 2000, only 46 autochthonous malaria cases were detected. Despite the fact that the malaria status of the Russian Federation remains rather stable, special attention must be paid to epidemiological surveillance on the disease. This is ever crucial in view of the ongoing importation of malaria from Tajikistan and Azerbaijan, sources of 350 (51,8%) and 305 (45,1%) cases respectively in 2000. During 1998-2000, cases of malaria have been reported in 30 regions, territories and republics of the Russian Federation.

An. messeae, *An. maculipennis*, *An. atroparvus*, *An. sacharovi*, *An. hyrcanus*, and *An. superpictus* are amongst the mosquito species of epidemiological importance in the Russian Federation. *An. melanoon*, *An. beklemishevi*, *An. claviger*, *An. algeriensis* and *An. plumbeus* are also registered. Some of the species have a record of DDT-resistance or are at the initial stage of resistance to malathion and propoxur. So far, no resistance to synthetic pyrethroids has been reported.

Generally speaking, antimalarial preventive interventions are carried out in potential foci in which introduced cases of malaria are confirmed. When local transmission is discovered, epidemic control interventions are carried out in the new active foci. In 2000, such foci appeared in 16 regions. Interventions performed included active case detection, radical treatment of all cases, seasonal chemo-prophylaxis with chloroquine, indoor insecticide spraying, entomological surveillance, treatment of breeding sites with bacterial preparations, health education amongst the population, and professional training.

A shortfall in the levels of funding provided to the health care system, including the units directly involved in malaria prevention and control, along with the large annual migratory inflow of population (around two million people from Azerbaijan, one million from Tajikistan, and tens of thousands of residents of other former southern republics where malaria has become a problem), hamper resolution of the problem at country level.

The WHO Collaborative Research Centre on *Plasmodium vivax* of the Martsinovskiy Institute of Medical Parasitology and Tropical Medicine, in collaboration with WHO (EURO and HQ), carries out organizational activities including the publishing of informational bulletins on malaria and its control/prevention in countries of the WHO European Region. In 2001, taking into account the complicated status of parasitological services and the difficulties in training staff, the WHO European Regional

Office provided financial assistance for the training of epidemiologists, parasitologists and entomologists at central and regional levels. Technical publications related to medical entomology are now under preparation by experts within the Centre in close cooperation with the RBM Unit of the WHO European Regional Office.

3.2 The Regional Roll Back Malaria Programme: Implementation results and future prospects

M.Ejov

Despite vast investments and strenuous efforts, malaria was never totally eradicated within the southern frontiers of the former USSR and Turkey. Throughout the 1970's and the beginning of the 1980's, epidemic malaria outbreaks were registered in the Ahsuin, Kjurdamir, Satlin, Imishlis and other districts of Azerbaijan. In 1977, several malaria epidemics engulfed the Adana, Ichel and Hatai provinces of Turkey, with the list growing to include the Adana, Osmani, Sanlurfa, Mardin, Adiana and Icel provinces from 1980-1986. In Tajikistan, several outbreaks were registered in the Kalai-Humb district in 1971, and in the city of Kuliab in 1978. Over 1981-1983, an epidemic rise in malaria morbidity embraced the Pianj, Parhar and Moscow districts, all of which border Afghanistan.

Though epidemic outbreaks of malaria were contained, it proved impossible to achieve the complete interruption of malaria transmission in the countries in question. At the beginning of the 1990's, the residual reservoir of malarial infection, aggravated political and socio-economic situation, mass population migration, deployment of extensive developmental projects, and nearly discontinued activities on malaria control and prevention had constituted the conditions favorable for the re-establishment of the transmission of malaria. As a result, large-scale epidemics broke out in Central Asia and countries of Trans-Caucasus.

Throughout the 1990's, large-scale epidemics of malaria were reported in Turkey, Tajikistan and Azerbaijan, while Armenia and Turkmenistan faced smaller-scale outbreaks. Over the past few years, the malaria situations in Georgia and Uzbekistan have also significantly deteriorated. Sporadic malaria cases have been registered in Bulgaria, Kazakhstan, Kyrgyzstan, Republic of Moldova, Belarus, Greece and Italy. The peak number of autochthonous malaria cases in the European Region (90,712) was registered in 1995.

In face of these large-scale malaria epidemics and the resurgence of malaria in the countries of the European Region, the WHO European Regional Office began to render financial and technical assistance to malaria-affected Member States. Thus, a regional strategy on malaria control was elaborated and adopted in 1999. The main targets of "Roll Back Malaria" are as follows:

- Prevention of malaria mortality in malaria-affected countries of the WHO European Region,
- Reduction in the number of *Plasmodium falciparum* cases and interruption of its transmission in Tajikistan,

- Prevention of the resurgence of *Plasmodium falciparum* transmission in malaria-affected countries of the WHO European Region,
- Halving malaria morbidity rates within the malaria-affected countries of the European Region by 2005,
- Maintenance of the epidemiological well being of countries within the WHO European Region in which malaria was eradicated.

The ultimate goal of the Regional Roll Back Malaria Programme is to interrupt malaria transmission, particularly *Plasmodium falciparum*, in countries of the WHO European Region by 2010.

Over the past several years, the malaria-affected countries of the European Region have revised their national strategies and programs in accordance with the concept and principles of the "Roll Back Malaria" global and regional movement. To this end, a number of regional and national-level meetings in Uzbekistan, Tajikistan, Azerbaijan, Turkey and other countries took place from 1999-2001. As a result, the leaders of the majority of countries confronting the resurgence of malaria have committed themselves to taking all possible measures aimed at containing malaria epidemics and outbreaks.

The WHO Regional Roll Back Malaria Program provides continuous financial and consultancy support to national health care services. This includes encouragement of partner and donor participation in the funding of malaria control programs, staff training at different levels, reinforcement of national health care services engaged in malaria prevention, treatment and epidemiological surveillance, promotion of research activities, and health education on malaria prevention amongst the population.

Serious commitments on the part of Member countries in the area of malaria control, continuous support from WHO (HQ and the European Regional Office), the high level of attention which has been drawn towards the problem, broad partnerships within the regional Roll Back Malaria Program, and a substantial increase in financial assistance according to country needs and local conditions, have together contributed to a significant reduction in the number of malaria cases in the Region. From 1995-2000, the number of autochthonous malaria cases reported decreased by nearly two thirds, or from 90,712 to 32,784.

The reduction in malaria morbidity was a result of joint efforts of the WHO European Regional Office and Ministries of Health of the member states. With the direct financial assistance of WHO, international and private institutions and donors, countries were supplied with essential diagnostics and laboratory equipment, reagents, antimalarial drugs, insecticides and indoor spraying equipment, transport facilities, computers, and other crucial supplies.

Over the last five years, the Regional Roll Back Malaria partnership movement has included all of the malaria affected countries of WHO Regional Office for Europe, International Federation of Red Cross and Red Crescent Societies (Azerbaijan and Armenia), UNICEF (Tajikistan, Azerbaijan, Armenia), UNDP (Azerbaijan, Armenia, Turkey), World Bank (Azerbaijan, Turkey), European Community Humanitarian Office (Tajikistan), WFP (Tajikistan, Armenia), USAID (Tajikistan), the Project Development Administration

Guneidogu Anadolu Projes (Turkey), the Italian oil and gas company Eni (Azerbaijan), non-governmental international organizations MERLIN (Tajikistan), ACTED (Tajikistan), MSF-Belgium (Azerbaijan), and donor countries including Norway (Armenia), Japan (Tajikistan), and Italy (Tajikistan, Armenia).

Within the WHO European Region, special attention is paid to managerial and technical staff training. Over the past two years, two international workshops on the planning of malaria control interventions have taken place in Uzbekistan. In June 2001, a Regional Study Tour on Malaria and its Control for Selected Member States was conducted in Tajikistan. Health professionals from Armenia, Uzbekistan and Turkey have undergone professional training abroad. Numerous training workshops for various categories of health personnel and entomological services staff have been carried out. Substantial effort was directed towards the preparation and publication of practical guidelines on malaria and its control.

The coordination of malaria control interventions, both amongst the countries of the European Region themselves and those countries which border them but are member states of the WHO Office for the Mediterranean Region, has been particularly emphasized of late. Coordination-related issues were repeatedly discussed at the regional meetings of directors of malaria control programs which took place in 1999 and 2000 in Azerbaijan. Taking into account the deterioration of the malaria situation in border areas of Tajikistan, Uzbekistan and Kyrgyzstan, the WHO European Regional Office, in collaboration with the Ministries of Health of the countries in question, initiated and conducted a trilateral meeting on malaria control in border areas. At this meeting, which took place in June 2001, it was recommended that all necessary measures should be taken to improve coordination among the countries in order to resolve common issues related to malaria prevention and control in Central Asia. It was decided to conduct such meetings amongst both the countries of the European Region themselves, as well as those bordering them, on a regular basis.

Despite the evident progress in containing malaria epidemics in the European Region, the epidemiological situation remains tense. Above all, this concerns the occurrence and spread of *Plasmodium falciparum* in Tajikistan, and the further spread of *Plasmodium vivax* within Central Asia. When considering that over 65% of Turkey's population resides in malaria-endemic areas and districts at high risk of the resurgence of malaria transmission, it is obvious that the prevention of epidemic situations will remain an urgent task for years to come. The drastic deterioration of the malaria settings in Georgia and Uzbekistan also calls for immediate action. Finally, the maintenance of the epidemiological well-being and successes attained in Azerbaijan, Armenia and Turkmenistan will require significant additional efforts.

4. Presentations

4.1 Some little-studied issues on taxonomy, biology and ecology of malaria vectors and their control in countries of the WHO European Region

M. Artemiev

For the most part, this review concerns malaria vectors in countries of the former Soviet Union. Rather than dwell upon success achieved, we shall consider the research and organizational problems faced by entomologists.

Taxonomy

After Bullini's work (Bullini et al, 1980) was published, the problem of *Anopheles subalpinus*, which for a long time had been considered synonymous to *An. melanoon*, came again to the forefront. The above-named authors have used the D indicator, i.e. a genetic distance. Having studied locuses of 27 enzymes, they discovered that *An. subalpinus* was closer to *An. messeae* (D=0,12), than to *An. melanoon* (D=0,15) and *An. maculipennis* (D=0,16). Thus, for *An. subalpinus*, a species denomination was suggested. Cianchi et al (1981) proved the autonomy of *An. subalpinus* and its proximity to *An. messeae* and supported the isolation of *maculipennis* sub-genus as had been formerly suggested (Buonomini & Mariani 1953). Later, they (Cianchi et al, 1987) studied large series of *An. subalpinus* from southern Yugoslavia, close to the site where this type of species had been discovered. The studies of 30 enzyme locuses have revealed the presence of two forms of local mosquitoes, without overlapping between the forms. One of them is similar to *An. melanoon* (including mosquitoes from Massarose, Italy, where the type was discovered), while the second presumably belongs to *An. subalpinus*. The taxonomic status of the relation of the latter form to *An. messeae* must be confirmed. It may be the case that *An. subalpinus* are merely southern populations of *An. messeae*, and such assumptions have already been expressed. Indeed, one can see while considering the melanoon-subalpinus habitat that, in contrast to habitats of many other species, it appears fragmented. In western (Cholchis) and eastern (Lenkoran) Transcaucasia, i.e. beyond the *An. messeae*'s habitat, numerous exophylic mosquitoes (probably *An. melanoon*) can be found. To the north of the Caucasian Range (Kabarda, Dagestan, the Kuban river reaches, Dnepropetrovsk and Donetsk areas, Crimea), some single low-number populations occur. Such mosquitoes are rare in these areas, and there is a chance that they were determined by melanistic eggs found in *An. messeae* and in *An. atroparvus* (Beklemishev 1937, with reference to Rubo and Prendel). As this issue is largely unexamined, one should start with studies of the Lenkoran and Cholchis populations.

Other little-studied issues worth mentioning include the establishment of the northern border of *An. maculipennis* in Russia and altitude limits of this species in the Caucasus and Middle Asia. It is also desirable to determine the southern borders of the *An. beklemishevi* habitat and *An. atroparvus*' eastern and northern borders. These issues are not only interesting from the theoretical standpoint, but are essential in view of global warming, when the northern borders of the southern species may shift by hundreds of kilometers, and habitats of the northern species decrease.

In consideration of species within other groups, the possibility of *An. petragrani* being a sibling of *An. claviger*, found to the west of the former USSR, remains strong.

The European and Middle Asian members of the *An. hyrcanus* complex of species, oriental by background, are poorly studied. The Far-Eastern form of this species had already been defined as *An. sinensis* by polythenic chromosomes (Gordeev, Klein 1997). However, Mudjiri (1941) had already marked the presence of at least two species in the Far East.

Disputable as well is the presence of *An. habibi* in the south of Middle Asia. In this case, the difficulties relate to the absence of an original description of this species.

Besides these particular species-related problems, truly bewildering is the tradition of "mosquito taxonomists" to heap up the subsidiary, absent in the International Code of Zoological Nomenclature (ICZN) categories. Thus, the rank below sub-species is sub-divided into numerous series to which Latin names are allotted, as in genera and sub-genera. For example, within the *Anopheles* sub-species, the following series in Southern Asia are listed: *Anopheles* s.str., *Lophoscelomyia* and *Myzorhynchus*, and within the *Cellia* sub-species, the series: *Neomyzomyia*, *Myzomyia*, *Pseudomyzomyia*, *Paramyzomyia* and *Neocellia* may be found. Moreover, these series are often subdivided into groups. For example, the *Myzorhynchus* series includes the groups "hyrcanus," "barbirostris," and "umbrosus." When it is difficult to define species by morphological features, and bio-chemical methods such as polythenic chromosomes, electrophoresis, etc. are brought in to resolve the issue, it is hard to understand the basis for the category of a "complex". Nobody can truly explain the difference between a "complex", "group", "sub-group", or "series"; nevertheless, the tradition continues.

There is an urgent need to determine the morphological features of different species in the "maculipennis" group, especially among its females, as they serve as the vectors. A number of species are well-distinguished by exochorions and swimmers of the eggs. Differences in larvae head pigmentation (but not among all species) have also been discovered (Yasiukevitch 1991-2001).

Ecology, life cycle and infectiousness

New studies on temperature thresholds and optimum ranges of the species of the "maculipennis" group need to be carried out. Data on the optimum of 25-30°C for the larvae of nearly all the group's species needs to be revised, as conflicting evidence has accumulated.

Also desirable is to continue the studies of species with a regard to antropophily and the factors which attract mosquitoes to humans and animals. Apparently, the host's body heat and the carbonic gas put out are not the only factors.

Studies on the repeated feedings of mosquitoes within a gonotrophic cycle are essential. This issue is of utmost importance from the standpoint of the epidemiological role of species.

Entomologists should apply immunological methods for the detection of infected mosquitoes. These methods are time-saving and allow for the precise detection of the species of malarial parasite. It is high time, too, to resume autopsies for the establishment of the physiological age of mosquitoes.

Chemical control methods

By the end of the 1980's, the monitoring of mosquito resistance to insecticides within the territory of the former USSR had been discontinued. By the end of the 1990's, with the assistance of WHO, some countries have resumed trials on mosquito susceptibility to insecticides.

The application of chemical insecticides for larval control is hazardous, and has no prospects. Neither could the use of chemical sprays be considered promising, given their ecological adversity, low effectiveness and high cost.

Nowadays impregnated mosquito nets, curtains, and screens are very popular. In principle, there are no objections to their use, but their main shortcoming is high cost. This prevents their mass application to personal protective equipment, though in vector control of exophilic (*An. hyrcanus*) and semi-exophilic (*An. pulcherrimus*) species, these means could take on a leading role, should the price prove acceptable. In Tajikistan, however, malariologists have already faced the problem that local people tend to go to bed only after the peak activity of vectors, which leaves them exposed to mosquito bites.

In the coming decade, the main control method of endophilic mosquitoes will remain the application of insecticides with residual action. Control of the quickly emerging irritability is quite possible by use of rotation of insecticides.

Biological control methods

Among biological control methods, only two are currently employed-entomopathogenous bacteria and larvivorous fish.

Among bacteria, the *Bacillus thuringiensis israelensis* serotype H-14 (Bti) against *Anopheles* and *B.sphaericus* against *Culex* are extensively used. The application of different Bti agents is limited by the fact that they sink rapidly, while the malarial mosquito larvae feed mainly on the water surface.

It may be the case that bacteria efficacy will be promoted by the creation of symbiotic bacteria cum infusoria systems. In this area, Dr L. Ganushkina is conducting quite promising research at the Martsinovskiy Institute in Moscow. She uses *Tetrahymena pyriformis* infusoria with Bti, *B.sphaericus* and *Methylobacillus flagellatum* with built-in Bti-creating genes. Recently, two graining *Brevibacillus laterosporus* strains have been isolated. In laboratory trials conducted to date, the infusoria had significantly increased the bacteria's action.

The use of floating forms of bacterial agents will be effective only if the toxin is protected against the sun's ultraviolet rays.

Larvivorous fish represent the oldest and the most effective of biological control methods; unfortunately, they are put to use only rarely due to the lack of knowledge concerning this type of fish amongst malariologists. This often proves the case with entomologists as well, who deal strictly with mosquitoes and their larvae. At present, the author of this article is completing a manual on larvivorous fish, with the following conclusions:

- 1) In the South, where *Gambusia* breeds and is actually a local species, it is entirely possible to organize its rearing and yearly distribution to anopheline ponds (including paddies). The *Gambusia*'s effectiveness has been established years ago.
- 2) In the North, where *Gambusias* and *Guppies* do not survive the winters, the following methods of larvae elimination may be suggested:
 - The use of local fish, primarily *Leucaspilus delineatus*, or minnows (*Phoxinus phoxinus*), *Umbra krameri*, and, for some areas, *Percottus glehni* in combination with pike, *Esox lucius*, is suggested. There is also a wide range of other fish species which could be put to use in this manner.
 - The use of local small fish in combination with large phytophagous fish is proposed. Such a combination is effective in that the latter eats away the plants and weeds in which mosquito larvae reside, and small larvivorous fish may then consume them without any obstruction. The *Ctenopharyngodon idella* can be used as phytophag, and it would be desirable to test the effectiveness of local fish, including tench (*Tinca tinca*) and rudd (*Scardinius erythrophthalmus*).
 - Imported larvivorous fish can be used in northern regions only during the warm season, and they must be provided with conditions suitable to survive the winters, a scenario possible only in a limited number of cases (i.e., in water sources near power stations).

In many cases, it is desirable to combine the use of fish with the treatment of weeded parts of ponds with bacterial agents.

Absolutely unexplored in countries of the European Region is the use of the predatory Cyclops of Mesocyclops, considered effective in many countries.

4.2 The taxonomy of malaria vectors in countries of the WHO European Region

O. Mamednyazov

The term "taxonomy" is derived from Greek, with "taxis" meaning "arrangement or order; regime," and "nomos," referring to law. In its contemporary understanding, taxonomy is defined as both theory and practice of scientific systematization and classification, and represents the knowledge of collateral subordination of taxonomic categories (taxons), ranging from species to systematic realms. The main task of taxonomy is the creation of a rational teaching on taxonomic categories (ranks) and their subordination (hierarchy), which allows for the construction of a natural classification. The terms "systematization" and "classification" are based on taxonomy.

The term "systematization" is derived from the Greek word "sistematikos" (ordered, pertaining to a system). We subscribe to the following definition: systematization is a division in biology aimed at the description and denomination ("naming") of all existing and extinct organisms, along with their classification by taxons (groupings) of different ranks.

The last term in this group is "classification", comprised of the Latin "classis" (rank, class, group) and "facio" (I make). In its present-day use, this term denotes the distribution of the multiplicity of all living organisms within a certain system of hierarchically subordinated groups - taxons (classes, families, genera, species, etc.). Classifications may be artificial or natural. An artificial system denotes a system that is based on one or several randomly chosen features. The concept of an artificial system is much broader; furthermore, systems of such type are frequently constructed in relation to particular objectives. Natural, or phylogenetic, classification takes into account the totality of features intrinsic to classified living objects. This makes it possible to either group them together or place them in opposition to one another, and it reflects historically established links among them. As a concept, "classification" is broader than "systematization".

In classification, a taxonomist employs 20 or more taxonomic categories, i.e. "species", "genus", "family", "order", "class", etc., all of different values and significance. Category refers to some rank or level of hierarchic classification, whereas organisms within a category are specific biological objects. Therefore, such concepts as "species", "family", etc., cannot be considered taxons, while the particular species, for example, *Anopheles messeae*, represents a taxon.

The categories naturally break up into following groups:

- Category of a species;
- Intra-specific categories;
- Higher categories.

All the taxons are historically authentic. Taxonomy issues are greatly diversified and in general relate to principles, methods and rules of classification. The current aim of evolutionary taxonomists concerns the creation of a natural (or phylogenetic) system. It is also assumed that such classifications are the most informative, diagnostic, stable and prognostic (Pesenko, 1989). Therefore observance of these principles is critical.

In addition to evolutionary trends, contemporary taxonomy also incorporates cladistic (phylogenetic) and numeric (phenetic) ones. In cladistic taxonomy, the rank of a taxon is determined according to sequence of isolation of separate branches (cladons) on the phylogenetic tree, regardless of the range of evolutionary modifications in any group. Thus, in cladistic theory, mammals do not appear as an autonomous class, but rather as a vermigrades-subordinated taxon. Numeric taxonomy resorts to mathematical treatment of the data based on a variety of randomly chosen features of organisms, allotting all of them the same value. The classification is founded on the measure of discrepancy between separate organisms, determined by different methods. The most important and extensively applied taxonomy method is that of comparative morphology. At the same time, new taxonomy methods, i.e. electron microscopy, karyo-taxonomy, hemo-taxonomy, geno-taxonomy, etc., are also used. Of high importance for animal taxonomy are different behavioral (etiologic) traits that at times may characterize specific features much better than structural differences (i.e. within the *maculipennis* group). The application of modern methods and in-depth research on population species structure has advanced taxonomy to a new stage of development. Taxonomic features serve for determination of taxons and are the indicators of kinship or similarity. Mayer (1971) emphasized four levels of application for such indicators:

- Subspecies identification (geographic variability);
- Discerning of closely related species, sibling species in particular;
- Grouping of related species into genera;
- Establishment of higher taxons relationships, from families up to types.

All zoological classification is based on the appropriate assessment of taxonomic features (weighing). Weighing represents the most important and one of the most difficult objectives facing a taxonomist.

Leaving aside the general biological importance of taxonomy, this report attempts to analyze and stress the significance of taxonomic features of malaria mosquitoes in the countries of the European Region.

Within malaria mosquito classification attempts to date, a system for the description of morphological features for each developmental phase, with detailed illustrations of body structure, has been established (Shtakelberg 1927,1937; Monchadskii 1936,1951; Monchadskii, Shtakelberg 1943; Gutsevitch et al 1970; Dubitskii 1970; Kuharchiuk 1980; Danilov 1985 a,b; Mamednyazov, Ponirovskii 1992; Sibatajev, Gordeev 1993; Bates 1940; Dubose, Curtin 1965; White 1978, etc.). While morphological features are still employed for classification more than any other, an ever-increasing number of methods are currently coming to the fore. In particular, these concern the most difficult of sibling species. The implementation of new types of taxonomic features is regarded as one of the aspects of the so-called new systematization. These un-morphological features work in addition to, rather than as a substitute for morphological ones (Mayer 1971). Their critical advantage is that they can be used for verification of the results obtained from routinely applied morphological features.

Over the past 60 years, substantial progress has been achieved in the area of genetic, biochemical and hybridological analysis of malaria mosquitoes. The main test model has been a closely related complex of sibling species of *An.maculipennis* (Beklemishev 1944; Reingard Topchiev 1955; Stegnii 1976 a, b, 1979,1980,1991,1993; Stegnii, Kabanova 1976; Stegnii, Sichinava, Sipovitch 1984; Gordeev 1986; Kitzmiller 1976; White 1978 etc.).

The isolation of ecological, behavioral, geographical and physiological features of mosquitoes, as well as an establishment of their relationship to malaria pathogens, represents a significant step within classification.

The implementation of new methods was required for merely practical purposes - in order to obtain precise data on the role of particular sibling species in malaria transmission, clarify the differences in their breeding sites and to work out effective methods of reducing the female, human-feeding populations. After all, efforts to combat mosquitoes of indeterminate species have never proven successful.

Hereafter, we will solely consider the morphological features of malaria mosquitoes.

Malaria mosquitoes have a prominent sexual dimorphism that manifests itself in their behavior and body structure, particularly in the morphology of the head and adnexa. The length and form of palpi vary and comprise the distinctive features that allow for the discernment of malarial vs. non-malarial mosquitoes, males vs. females.

The biological classification of malaria mosquitoes refers to the grouping of those organisms that are similar as a result of their common origin. The taxonomic features that determine those similarities are tegulae, thorns, setae, hairs, dimensions of different body parts, body coloration, presence on the body or wings of spots, stripes, etc. As taxonomic practice confirms, not all taxonomic features are useful. Essential are the key features - they are easily recognizable, have low variability, can be well preserved as samples, and may serve as useful markers of the taxons, determined during classification. A number of taxonomic features, i.e. chemical, chromosomal and physiological, are rather valuable for classification ends, but of are of little use or even useless for determination of species, because they are difficult to detect within a sample or require the application of complicated assessment methods (Mayr, 1971). Thus, it is worth mentioning that traditional morphological analysis in the taxonomy of malaria mosquitoes maintains the leading role.

The Anophelinae sub-family is comprised of three genera. The Anopheles genus species are prominent in the South Arctic area. The Anopheles genus subdivides into seven sub-genera. In the South Arctic area, including the countries of the WHO European Region, the species of Anopheles Mg. and Cella Theob sub-genera are typical.

Each phase of the development of Anopheles will now be explored.

Imago

The key taxonomic features of Anopheles sub-genus (by females) are as follows: traverse fibres and fork's base r_2+r_3 , m_1+m_2 and $m_{3+4}+cu_1$, covered by dark tegulae; the front border of costal fibre is covered by monochromatic dark tegulae, or has not more than two light-colored spots.

For ease in determination, we have subdivided the imago of the Anopheles sub-genus into three groups. The first group is comprised of all eight species of the South Arctic "maculipennis" complex, distinguished by dark spots on their wings. Among these, the spots on the wings of *An.sacharovi* and *An.martinius* are barely visible, and their mid-dorsum is monochromatic and of light fulvous color. Among the other six species, i.e. *An.messeae*, *An.beklemishevi*, *An.atroparvus*, *An.melanoon*, *An.maculipennis* and *An.labbranchiae*, the spots on wings are well pronounced, and the mid-dorsum has a wide grey stripe and dark sides. In contrast to all the other "maculipennis" group species, *An. maculipennis'* upper wing fimbria is light-colored.

The classification of the "maculipennis" complex represents one of the most complicated and contentious issues in mosquito taxonomy. To date, no valid morphological differences have been discovered among the members of the complex, neither among larvae and imago nor, in particular, in the structure of hypopygium. Lately, the morphological differences among the larvae of some "maculipennis" species (ovipositions being the only reliable distinctive feature) have been reported.

The second group is comprised of *An.algeriensis*, *An.claviger*, *An.plumbeus*, *An.barianensis* and *An.marteri* – all species without spots on their wings. *An.algeriensis* is easily distinguished by its absence of a bundle of white tegulae on the forehead, while a light-colored proboscis top characterizes *An.marteri*. The large spring mosquito *An.claviger* is easily identified by an off-white longitudinal strip on its mid-dorsum. Hollow

mosquitoes *An.plumbeus* and *An.barianensis* are characterized by small size and a blackish-gray color with a lead-like shimmer, as well as by the presence of a pronounced bundle of pure white color tegulae on the front side of mid-dorsum. *An.barianensis* differs from *An.plumbeus* in its more pronounced white tegulae on the upper part of its hips, along with a more evident bunch of white tegulae on the front spiracle.

The third group includes *An. hyrcanus* and *An. lindesayi*. They both have white spots on costal fibre. *An. hyrcanus* has two large white spots on the front side of its wing, one at the beginning of the distal third, and another before the wing's top. *An. lindesayi* has a single large white spot at the wing's tip that covers $r_1 + r_2$. There are also small areas of white tegulae on other fibres.

Anopheles sub-genus (by hypopigias of males)

The structure of hypopigias constitutes a rather important feature in the taxonomy of mosquitoes. In many cases, the structure of hypopigias accounts for the most accurate and reliable determination of a species, and sometimes it represents the only source for identification. Therefore, this feature is often considered the main interspecies criteria.

The key feature of the *Anopheles* sub-genus by hypopigias of males is as follows: coxitis along the base from interior side, generally with two (less often with one or three) strong setas, at least one of them located on the papillar tuberculus. The following feature easily distinguishes *An.algeriensis* and *An.claviger*: in the former, there is coxitis along the base from interior side with one strong thorn, and in the latter, the same feature, with three strong setas, two of them forked. All the other species of the *Anopheles* sub-genus show coxitis along the base from interior side with two simple strong setas. The clasp setas of *An.plumbeus* and *An.barianensis* (as in all the species of "maculipennis" complex) are not joined. These two groups differ by the structure of edeagus: in the former, it is short and wide, without adnexa. The differences between *An.plumbeus* and *An.barianensis* (as among the species of the "maculipennis" complex in general) are largely insignificant, with no reliable features allowing for their distinction as of yet discovered.

In *An.marteri*, *An.lindesayi* and *An.hyrcanus*, at least some of the clasp setas are joined, forming transparent laminae. *An.hyrcanus* differs from the first two by long blades of IX tergite. *An.marteri* and *An.lindesayi* differ by seta's width at the middle part of the coxitis; it is large in the former and small in the latter.

Cellia Sub-genus

The key taxonomic features of the *Cellia* sub-genus (in females) are as follows: traverse fibres and fork's base r_2+r_3 and m_1+m_2 are covered by white tegulae; the front border of costal fibre has four or more light-colored spots.

In the countries of the WHO European Region, three species of malarial mosquitoes of the *Cellia* sub-genus are found. *An.pulcherrimus* – a white malarial mosquito - differs from the two other species of the *Cellia* sub-genus by the white coloring of its three last segments of rear fibiotarsus, white tegulae bundles along the sides of abdomen, and snowy white tegulae on the body and wings. *An.multicolor* and *An.superpictus* have

dark tibiotarsus segments, abdomen without tegulae bundles, and grayish-cream coloring of wing and body tegulae. Between themselves, they differ in coloring of the top of the last palpi' segment - it is dark in *An.mubticolor*, and light in *An.superpictus*.

The key taxonomic feature of the *Cellia* sub-genus (by hypopigias of the male) is as follows: coxitis along the base, with five strong setas. The presence of leaf-like adnexa on top of edeagus in *An.superpictus* distinguishes it from *An.multicolor* and *An.pulcherrimus*, as they have no leaf-like adnexa on the top of edeagus. They are also different in cluspets structure, with no expansion of lamina on top in *An.multicolor*, and an expanded one in *An.pulcherrimus*.

Larvae

In the taxonomy of the *Anopheles* and *Cellia* sub-genera larvae, the main distinctive features are: 1) positional relationship, character of branching and development of the front and rear liner's hairs (2-,3-and 4-C), and frontal hairs (5-,6-and 7-C); 2) location and development of hair on feeler's body (1-A); 3) development and branching of middle hairs of prothorax (1-, 2-and 3-P), and side hairs of prothorax (9-12-P); 4) shape and development of palm-like hairs on metathorax (3-T), and, in particular, on abdomen (1-II-VII); 5) development of side hairs (6) on IV-VI abdomen segments; 6) development and branching of thorax and abdomen hairs on ventral side; 7) structure of stigmal blade and crista.

Within the determination of larvae, it is essential to study both their dorsal and ventral sides. Examinations should be carried out on IV stage larvae.

Short and simple frontal hairs characterize the larvae of hollow mosquitoes *An.plumbeus* and *An.barianensis* of the *Anopheles* sub-genus. All four clipeal hairs of *An.plumbeus* are located at a similar distance from each other, and in *An.barianensis* the distance between the 2-C bases is half the size of that between 2-and 3-C. There are other distinctive features among these species: in *An.plumbeus*, the 2-and 3-C hairs carry 4-8 side-lines, 1-P hairs are very branchy, pinnate, ventral side hairs of thorax and abdomen are well-developed and star-branched, which gives the larva a thorny look; in *An.barianensis* the 2-and 3-C hairs are simple, 1-P hairs less branchy, non-pinnate, and ventral side hairs of thorax and abdomen are poorly developed and simply branched.

The larvae of the remainder of the *Anopheles* sub-genus species and the larvae of all species of the *Cellia* sub-genus have long and pinnate frontal hairs. Larvae of the *Cellia* sub-genus have 2-C hairs set up at a distance, and simple and short hairs on feeler's body. The larvae of *An.pulcherrimus* from the same sub-genus are easily distinguished by branching of 3-C hairs (6-15 hairs), whereas the larvae of the two other species (*An.superpictus* and *An.multicolor*) have simple hairs. The latter species can be differentiated from one another by a number of differences. All or nearly all the *An.multicolor* larvae have 5-, 6-and 7-C hairs with dark spots around their bases, 1-P hairs are ill-branched, appearing from ill-pronounced tubercles, and the front part of the central blade of respiratory apparatus is long and narrow, often spear-shaped. The *An.superpictus* larvae, on the contrary, do not have dark spots around bases of 5-, 6-and 7-C hairs, 1-P hairs are well-branched and appearing from well-pronounced tubercle and the front part of central blade of respiratory apparatus is wide, short and rounded.

The Anopheles sub-genus larvae are defined by close placement of 2-C hairs and pinnate-branched hair on feeler's body. Furthermore, the larvae of *An.hyrceanus* and "maculipennis" complex have tree-like branched 3-C hairs. The *An.hyrceanus* larvae are distinguished by a straight 1-A hair not shorter than half the feeler's size in length and extending from around its middle. The "maculipennis" complex larvae feature a twisted 1-A hair of a length visibly shorter than half the feeler's size extending from a point a quarter of the way down from its base. It is worth mentioning that to date, no reliable taxonomic features of the "maculipennis" complex larvae have been established. Some species, though, may be distinguished from the others by their larvae, for the number and branching of hairs on adnexa and size and coloring of some body parts of larvae can be considered characteristic for each species of a given locality. For example, *An.sacharovi* and *An.martinius* have small and light-colored stigmal plates (0,38 - 0,5 mm long, with a distance between the ends of side blades of 0,39 - 0,52 mm). The other species of the "maculipennis" complex (*An.maculipennis*, *An.beklemishevi*, *An.atroparvus*, *An.melanoon*, *An.messeae*, *An.labranchiae*), have a large dark-colored stigmal plate (0,5 - 0,57 mm long, with the distance between the ends of side blades 0,56 - 0,59 mm). The distinction between the *An.beklemishevi* and *An.messeae* larvae may be based on the crista's structure, branching of interior clepial and prothorax side hairs, and branching of additional dorsal hairs on IV and V abdomen segments (Sibataev, Gordeev 1993). The *An.maculipennis* and *An.martinius* larvae have differing hairs on the second segments of their abdomens: while in *An.maculipennis* the branches are thin, in *An.martinius*, they are wide and palm-like, although smaller than the same hairs on the following abdomen segments.

The other species of this sub-genus (*An.algeriensis*, *An.claviger*, *An.marferi* and *An.lindesayi*) have simple or slightly branching 3-C hairs (either at the ends or from the middle). At that, 2-and 3-C hairs of *An.algeriensis* located on distal half or one third are with slight pinnation. The crista of stigmal plate have 20-25 teeth. The larvae of the other species have smooth 2-and 3-C hairs; 4-C hairs of *An.claviger* have 2-5 branches, and 4-C hairs of *An.marferi* and *An.lindesayi* are simple. The larvae of these two species differ from each other in the number of petals of palm-like hairs on the metathorax: 10-11 petals are found on *An.marferi*, and 15-20 in *An.lindesayi*.

Eggs

As we have learned from experience, studies of the eggs may provide taxonomic information. Reference is made to Mayr (1971): "According to data, malaria in Europe is transmitted by a single species of gnat - *An.maculipennis*. While studying the distribution and ecology of this species, researchers discovered a number of strange deviations. It was discovered that Anopheles mosquitoes were quite common in some malaria-free European areas. While in some regions, they feed only on domestic animals, in others, they prefer to feed on man. In some regions, they prefer sweet water, while in others they choose brackish. After Falleroni had discovered a stable difference in the biological features of the eggs, the situation started to clear up. Finally, it was established that the European gnat is actually represented by six groups of sibling species. Later, by means of cytogenetic research (Stegnii, Kabanova 1976), the new species *An.beklemishevi* was identified in Northern Eurasia, and the validity of *An.martinius*, described by N.I.Shingarev (1926), was re-established.

After 70 years of tenacious research, the most valid morphological differences among these eight species of the Anopheles sub-genus yet remain those found in the coloring

and structure of the eggs. The eggs of the "maculipennis" complex (except *An.martinius* and *An.sacharovi*) have air-filled floats located in the middle third of the egg. Structure and coloring of the external membrane, exochorion, vary, but is characteristic to many species. This depends on local modifications of the exochorion structure, and includes dark and light spots and stripes. Interestingly, no differences between *An.martinius* and *An.sacharovi* have been discovered to date. In conclusion, it is noted that up-to-species determination using microscopic slides of the Anopheles pupae has been carried out. However, since this method is rarely employed in practice, little attention has been devoted to it.

4.3 Results of cytogenetic research of malaria mosquito populations in countries of the WHO European Region

M. Gordeev

Despite the extensive application of molecular methods in biology, the cytogenetic approach retains its topicality in the research on the dynamics of malarial mosquito populations. To date, no molecular genetic markers for the sibling species of the Anopheles maculipennis complex have been detected. As before, the cytogenetic method represents the only reliable way of conducting diagnostics of South Arctic species of malaria mosquitoes. The aim of this report is to illustrate principal trends in the current research on malarial mosquito populations.

There are three groups of factors that significantly affect the dynamics of vector populations:

- Urbanization
- Regional eco-catastrophes
- Global warming

Response adaptive reactions depend on species composition and the ecological and genetic structure of the malarial mosquito populations. An important element of the genetic structure of the populations of a number of potential malaria vectors is the chromosomal variability.

In the analysis of chromosomal variability the paracentric inversions (a 180-degree turn in the chromosomal section) have been used as genetic markers. To register inversions, polytene chromosomes of the salivary glands of larvae and the malpighian vessels of imago have been studied.

In Europe, the main species are *An. messeae* and *An. maculipennis*. Their karyotypes have been studied in the Upper Rhine valley, where *An. claviger* and *An. plumbeus*, with precise ecologic specialization, were also found. *An. messeae* was the dominant species. Together with *An. maculipennis*, its larvae have been discovered in just a single biotope, open and well warmed. Urban species were detected in *An. Plumbeus*, which had acclimated not only to hollows in trees but to urban drainage and sewage systems, which raised the epidemiological importance of this species. *An. messeae* adjusts to man-caused conditions by inversion polymorphism. The variability of the XL chromosome for *An. messeae* populations in the Upper Rhine valley has been discovered.

Different ranges of chromosomal variability are observed in Eastern Europe. In the Moscow region, *An. messeae*, *An. maculipennis* and *An. claviger* are found. The larvae of the first two species reside in the same ponds. Near Moscow the ratio of these species may vary. Within Moscow city limites, *An. messeae*, whose karyotype carries 5 inversions, is the dominant species.

More to the South, in the Voronezh region, the presence of *An. maculipennis* reaches 93%, and the few *An. messeae* that reside there exhibit the same karyotypes as *An. messeae* in the Upper Rhine valley. In Dagestan (Kizliar district), only *An. maculipennis* has been detected.

In the Urals and Western Siberia reside *An. messeae* and *An. beklemishevi*. The latter dominates in the north of taiga zone, while in the Middle Urals and the south of Western Siberia this species is rare. *An. claviger* was not found in Western Siberia. Among chromosomal inversions of *An. messeae* in the Western Siberia, the "northern" prevail. In the last 15 years in the south of Western Siberia, the "northern" karyotypes of *An. messeae* and *An. beklemishevi* have been replaced by the "southern", more endophilic type.

The Zeya river basin of the Far East represents the southwestern border of the *An. messeae* habitat, and to the east, there is only "*An. hyrcanus*," which Gutsevitch has considered identical to the "western" form. A chromosomal chart was composed for *An. hyrcanus* from the Chui and Fergan valleys, where a high level of chromosomal variability had been detected. It was revealed that the "eastern" form had a specific chromosomal polymorphism. This form was defined as *An. sinensis*, based on its comparison with karyotypes of the Chinese and Japanese *An. sinensis*, and the morphology of the eggs.

In Northern Kazakhstan (from the northern ridges of Kirghiz range to Zailiisk Alatau), only *An. messeae* has been discovered. The chromosomal structure of its population significantly differs compared to the Siberian mosquitoes. In Southern Kazakhstan, it proved impossible to locate *An. martinius*, but *An. messeae*, *An. hyrcanus*, *An. claviger*, *An. superpictus*, and (for the first time) *An. maculipennis* have been detected. The chromosomal structure of *An. messeae* in the south of Kazakhstan is quite homogenous. The mosquitoes are fully homozygous by XLI and 2R0 inversions, just as in the north of Kyrgyzstan.

In the Chu river basin, *An. Messeae* serves as the dominant species, although *An. claviger* and *An. hyrcanus* are also found. *An. martinius* was not detected here (though it had been found by Bublikova in 1998). In the Tien Shan internal regions *An. maculipennis* dominates, and *An. claviger* is discovered in the Issyk Kul region.

In the Fergan valley and the adjacent areas (in addition to *An. pulcherrimus*, *An. superpictus*, *An. hyrcanus*) *An. Maculipennis* is one of the main vectors to have been detected. *An. martinius* was not found.

In the Amu-Darya valley, especially in Khorasm region and Kara-Kalpak, *An. pulcherrimus* and *An. superpictus* have been dominating. *An. martinius* is found in the Pre-Aral area.

4.4 The ecology of malaria vectors in countries of the WHO European Region

V. Anufrieva

An. maculipennis

An. maculipennis resides in European Russia, in the Caucasian Region, and in the foothills and mountains of Middle Asia. It is the main vector within the mountainous districts of the Trans-Caucasian Region.

The breeding sites of *An. maculipennis* include reservoirs of flowing water, and, less often, stagnant ponds in flood-lands and riverbeds. The normal vital activity of the larvae takes place at temperatures from 10 to 35° C, with 25-30° C considered optimal. Temperatures below 7.5° and higher than 35°C produce cold and heat stupor of the larvae. *An. maculipennis* larvae are found in ponds rich in oxygen and low in salinity. This species' larvae reside in small ponds and pebble beds of rivers and streams in the mountains without plants and weeds. The species has been reported at altitudes of 2000 m above sea level. Away from mountainous and hilly areas, the species disappears.

Adult mosquitoes are endophilic. They attack man both indoors and outside. The ratio of human blood-fed females depends on the numbers of cattle in local farms and the degree of exposure. Repeated blood-feedings within a gonotrophic cycle have been recorded. Females winter either in premises with a host, and then feed on blood throughout the winter (in low mountains and plains with warm winters), or in cold shelters far from host (high mountain areas). In warm districts, the diapause lasts for just 1 or 2 months, increasing to 7 or 8 months in areas higher up in the mountains.

An. messeae

An. messeae represents the species with the most extensive habitat. It is a main potential vector in the majority of areas of Russia, the Baltic region, Belarus, Ukraine, northern Kazakhstan and Kyrgyzstan.

An. messeae females start to fly out and attack when the temperature reaches 7° C; their upper activity threshold is 25-30° C. At 25° C they still actively attack, but never at temperatures above 30° C. The upper temperature threshold of adult mosquitoes is 35° C. In summers, optimal temperatures of their resting sites by day reach 19-21° C. This is a hygrophilous species that by day avoids dry resting sites in which air humidity is lower than 65%. This would seem to serve as the main preventive factor for the distribution of this species farther south. The *An. messeae* females are to a certain extent endophilic, and they tend to form clusters in granaries, cellars, sheds and even in dwellings. Their host is usually cattle, but attacks on man are also frequent.

It has been established that within the taiga zone two generations of this species develop in one season; in the southern part of a forest zone, 2-3 generations; in the forest-steppe zone - possibly 3-4 generations; in steppe zones - 4-5 generations; and in Middle Asia and the Trans-Caucasian lowlands, 6-7 generations.

The females winter in cold premises, far from hosts. The diapause is profound, and during winters, mosquitoes never feed on blood. The first diapausing females in the

northern reaches of their habitat emerge by the end of July, achieving the greatest numbers in population by mid-September. In the spring, they emerge in flight from different premises within a period of time that sometimes lasts for one or two months, depending on the speed at which their premises have warmed up. Fly-out periods vary from year to year, from mid-April to mid-May, and occur when the mean daily temperatures reach 4-5° C. Observations of *An. messeae* have called forth the appearance of the term "malaria-free anophelines." The abundance of these mosquitoes within their day-resting sites in Northern Europe and Siberia was contradictory to the absence or low malaria morbidity in these areas. One proposed reason for this is the low temperatures within the areas mentioned above. For example, at Vologda's latitude, the temperature is high enough for completion of a single sporogonic cycle in the mosquito's body only once every 3-4 years. At Moscow's latitude, the cold summer makes malaria transmission impossible every 3-4 years.

The susceptibility of *An. messeae* to malaria parasites, its contact with man, and the number and survival of this species bring together conditions sufficient for malaria transmission. This fact has been proven repeatedly by experience, when some years ago *An. messeae* served as the cause of large malaria outbreaks in the USSR.

An. atroparvus

An. atroparvus is a plains species breeding in saline water reservoirs. It is distributed to the west of the Riga-Astrakhan line. The northern border of the habitat of this species is found approximately along the straight line from Kaliningrad in the west to Astrakhan in the east. The species cannot survive in cold winters or too-hot summers. It is absent in the Trans-Caucasian Region. This species is also one of the main potential malaria vectors.

The larvae develop in stagnant, slightly saline waters, usually with aquatic vegetation. The temperature demands of the larvae are the same as for *An. maculipennis*'s.

The species is endophilic, and attacks both indoors and outside. Males pursue females everywhere, so mating can also occur indoors. Due to this fact, the species is easily cultivated under laboratory conditions. Females prefer the blood of large animals, but at the same time, willingly feed on man.

The wintering of *An.atroparvus* takes place only in comparatively warm premises, in districts with mild winters, and their day resting site temperature varies from 3 to 9° C, with some decreases to 0° C. Fat deposits of the diapausing females are quickly spent in August. The emergence from diapause is gradual and takes place in accordance with warming. V.N.Beklemishev (1949) noted a case in which the ratio of infected females among wintering mosquitoes reached 20-30%. In districts with milder climates, *An.atroparvus* is not an effective vector. Nevertheless, in some regions of Ukraine, Crimea and Northern Caucasus, this species has supported persistent malaria foci.

An. beklemishevi

An. beklemishevi is a northern species distributed within the plains and highlands of European Russia and western Siberia. Its role in malaria distribution is unstudied. While several researchers had previously recognized this species as *An. maculipennis*, a species with a similar distribution pattern, studies on polythene chromosomes have

revealed the fact that in the western Urals, Urals and the north of European Russia, another species is distributed as well, differing in its egg structure (V.N.Stegnii, V.I.Kabanova 1976). Ecologically, this species is close to *An. messeae*. Much of the previous data on the ecology of *An. maculipennis* within the northern reaches of its habitat in fact refers to *An. beklemishevi*. The significance of this species in malaria distribution is the same as *An. messeae*.

An. sacharovi

An. sacharovi is a thermophilic species of the plains that often resides in saline reservoirs. It is prevalent in the plains of the Trans-Caucasian Region and in Dagestan. *An.sacharovi* Favre represents the most southern and thermophilic species of the "maculipennis" complex amongst all species of the European Region. It is the main malaria vector in Azerbaijan, and extremely anthropophilic.

The species resides in a great variety of water sources, from filtration puddles to stagnant water pools to drainage ditches, as well as other waterlogged areas, near rivers, and in other areas. These places usually contain vegetation. The *An.sacharovi's* larvae may develop in highly saline waters and can sustain seawater admixtures.

The adult mosquitoes are thermophilic and begin to fly out only when the temperature is higher than 10° C. *An.sacharovi* are hydrophilous, strictly endophilic, and do not have day resting sites in nature. They attack man both indoors and outside. If cattle are present, some of the mosquitoes will feed on them. Repeated blood feedings within a gonotrophic cycle have been recorded, which significantly increases its epidemiological role. Mating occurs both indoors and outside. The diapause starts at the end of September, but mass diapause of the females takes place one month later, and usually finishes by February. During just one season, up to 6-7 generations may breed. Seasonal population levels vary from district to district. In districts with many constant water sources, mosquito numbers may remain high throughout the summer and the first half of autumn. Finally, the last type of seasonal numbers dynamics occurs in cool districts with numerous breeding sites, most commonly in the foothills. At the beginning of summer, after wintering, mosquitoes are nearly absent; then, as a result of the reproduction of single numbers of local mosquitoes and migration from neighboring districts, the numbers increase, reaching their peak in September. Thousands of mosquitoes set off for wintering, but very few survive.

An. sacharovi is considered the most important malaria vector in the European Region.

An. martinius

An. martinius is a plains species distributed in the north of Middle Asia and the south of Kazakhstan. Until 1978, this species had been considered a Middle Asian form of *An. sacharovi*. Data obtained following the crossing of this form with Trans-Caucasian mosquitoes revealed an unproductive isolation (V.N.Stegnii 1980), and the name "martinius" originally given by N.I.Shingarev in 1926, was returned to this Middle Asian form of the species. *An. martinius* is abundant in Uzbekistan and the north of Turkmenistan and Tajikistan, and may also be found in the south of Kazakhstan. In Kazakhstan, it is distributed in areas up to latitudes of 46° North. At present, the numerous findings of the species in the south of Turkmenistan, Uzbekistan and Tajikistan

have not been confirmed. It is likely that even if the species was present there, it would have disappeared in the hottest of districts following DDT spraying.

The larvae of this species can normally develop in ponds in which the day temperature rises to 38–40°C. The larvae are found in various water sources including filtration puddles, stagnant water ditches, drainage ditches, waterlogged areas, rice paddies, and other areas. These pools almost always contain aquatic vegetation. The larvae can sustain high salinity. Adult mosquitoes are thermophilic. Some exophily is also recorded. The mosquitoes attack man both indoors and outside. In general, they feed on cattle, but readily attack man as well. They winter similarly to *An. messeae*, in cold premises, without blood-feedings. The diapause starts at the end of September, very quickly embracing the entire population, and ends in February. Within one season, 5–6 generations may develop. The species transmits malaria less effectively than *An. sacharovi*.

An. hyrcanus

An. hyrcanus in Europe resides south of 50° latitude, and is common in the Trans-Caucasian Region, Kazakhstan and Middle Asia. It is exophilic and widely distributed in rice cultivating districts. While the species is a carrier of *Plasmodium vivax*, it cannot transmit *Plasmodium falciparum*.

Optimum temperature for the larvae is 25–30°C, the same as for the majority of the species of the “maculipennis” complex, but the lower temperature threshold of their development is 12°C, and the higher is 35°C. *An. hyrcanus* prefers temperatures neither too high nor too low; in the northern reaches of its habitat, it populates the most sun-heated ponds, whereas in the southern districts, it is found in shadowy areas. In cold districts, in conjunction with the slow heating of water sources, the larvae's development is slow in the spring, with an abundance of these mosquitoes appearing later in July. In Middle Asia, *An. hyrcanus* in mass appears at the end of May - beginning of June, with its numbers decreasing by August and rising again in September-October. The species' preferred water sources include surface feeding swamps, floodlands, river bays, dry riverbeds and rice paddies, usually those with abundant aquatic vegetation.

The adult mosquitoes are hydrophilous and exophilic, with day resting sites outdoors, including grass, bushes, on the banks of irrigation canals, holes in fencing, etc. In Middle Asia, they may at times be found inside living quarters. The species may change the type of day resting site according to air temperature, but usually remains exophilic. It attacks man both indoors and outside. In literature, *An. hyrcanus* is described as a small hunter of a host, but in Middle Asia and Kazakhstan, incidences of massive mosquito attacks on man within settlements have been recorded.

The diapausing females winter in natural shelters including clefts in the ground, reed bushes, etc. In warm winters, they fly out for blood meals, but even in Middle Asia, this is rare. In the south of Middle Asia, the diapausing females appear at the end of September, and their numbers become abundant in October. The last attacks of gonoactive females on man are recorded in mid-September. In colder districts, the diapause starts a bit earlier. *An. hyrcanus* is considered a secondary malaria vector. Within the intense malaria foci of the above districts, *An. hyrcanus* is accompanied by other Anopheles species which present themselves as the main malaria vectors. For example, in Middle Asia, this is *An. pulcherrimus*. In the Far East, where *An. hyrcanus*

represents the only possible malaria vector, outbreaks of malarial disease have occurred only rarely and have never reached large dimensions. It has been established that the vector in these cases was *An. sinensis* rather than *An. hyrcanus*.

An. superpictus

An. superpictus is distributed in the mountains and foothills of Middle Asia. It is found in the Trans-Caucasian Region, Dagestan (to the south of Mahachkala), to the south of Sochi and along the southern coast of the Crimea. It breeds in mountain riverbeds in calcium-rich waters of high salinity. In the mountains of Middle Asia, it is found in elevations as high as at 2800 meters above sea level. This species serves as the main malaria vector in the mountain and foothill districts of Middle Asia.

The larvae reside in shallow pebbly ponds in mountain riverbeds. The water in such ponds is rich with calcium salts. Vegetation usually consists of not more than filiform water-plants. Water sources are either stagnant or slow-flowing. In the daytime, temperatures rise up to 35–38°C, with 30°C representing the optimal warmth. At such temperatures, larval development takes 11 days; at 20° C, 24 days must pass. *An. superpictus* larvae are also found in other types of ponds including rice paddies, the crucial element being a high content of calcium salts. The ecological demands of the larvae defines the geographical and landscape distribution of *An. superpictus*. The best conditions for this species are found in the mountains, where summers are hot and dry, and rivers during these times dry up, leaving numerous pools suitable for breeding. In the Caucasian Region, with its humid climate, frequent showers and comparatively low summer temperatures, the numbers of *An. superpictus* are low, and mountain districts are mainly populated with *An. maculipennis*. In the mountains of Middle Asia, *An. superpictus* may be found at elevations as high as at 2800 meters above sea level.

The adult mosquitoes are thermophilic and xeric, highly exophilic, and find favorable settings for resting by day in warm premises and outbuildings. They attack their victims mainly indoors (endophags). The mosquitoes are capable of covering long distances in search of hosts and therefore tend to accumulate within settlements. They are highly active and mobile when attacking. The diapausing females usually winter in cattle-sheds in close proximity to hosts. In the Alasan valley in Georgia, though, they can also winter in vegetation, tree hollows and rodent holes. The species feeds on blood throughout the winter, and stops its activity only at the coldest of times. The mass appearance of diapausing females is observed in October. Fly-out from wintering sites takes place in March and April. During spring and the first half of summer, *An. superpictus* numbers are insignificant, because at these times the ponds suitable for breeding remain few: riverbeds are full of water flowing from the mountains, and ponds in pebbly riverbeds are nearly absent. By mid-summer the water levels in rivers subside, and riverbeds dry up, allowing for the formation of numerous ponds suitable for *An. superpictus*. From this time onwards, *An. superpictus* numbers steadily grow, reaching their peak in September-October. Unfavorable conditions for *An. superpictus* occur both in years with many summer showers, when water levels in rivers increase and breeding sites cannot be formed, as well as during drought years, when water levels decrease considerably and breeding sites dry up. *An. superpictus* numbers reach their peak only in years with average water levels in mountain rivers. Thus, the numbers of this species undergo significant fluctuations from year to year, depending on meteorological conditions. Though this circumstance decreases the epidemiological significance of *An. superpictus*, it remains one of the main malaria vectors in the

mountain and foothill districts of Middle Asia and some Georgian districts (G.I. Kanchaveli 1957). The species is easily infected by *Plasmodium vivax* and, especially, *Plasmodium falciparum*. As it has been already noted, the epidemiological significance of this species is to some extent decreased by drastic fluctuations in number from year to year; nevertheless, these fluctuations may be held accountable for sudden large outbreaks in districts which were formerly considered safe.

An. pulcherrimus

An. pulcherrimus is a plains species residing in Middle Asia and the south of Kazakhstan. It is often found in saline waters and rice paddies. The species is semi-exophilic, and it serves as the main malaria vector in the Middle Asian plains.

The larvae develop in well-heated stagnant ponds, usually with dense vegetation. Rice paddies may also constitute highly suitable conditions for larvae development. While optimal water temperature is at 30°C, the larvae can sustain temperatures of up to 35°C. Decreases to below 25°C suppress larvae development. *An. pulcherrimus* larvae are resistant to water pollution from nitrogenous substances, and can develop in saline waters. Adult mosquitoes are suited to life in the hottest and most desert-like areas. The white colouring of the body and wings contribute to reflection of the sun's rays, and narrow spiracles prevent water evaporation as they breathe. This species is the most thermally stable and xeric mosquito of the fauna of the European Region. Females are not afraid of sunlight, and can sustain exposure to direct sunlight at high temperatures. As day-resting sites, *An. pulcherrimus* prefers open cattle-sheds, granaries, clay-walled fences, bushes, grass, dried up ditches, pits, and similar environs. They avoid day resting sites in highly humid and shaded places. The choice of day resting sites defines the semi-exophilic mode of life of these mosquitoes. The species attacks man and cattle at temperatures of 12–33° C, and is most active at 19–28° C. On warm nights, the mosquitoes are active from dawn until sunrise, whereas on cold nights they remain active only during the evenings and mornings. As they prefer large gatherings of hosts, they tend to accumulate in settlements. Females of this species may travel long distances in search of a host. In the south of Middle Asia, these mosquitoes appear in mass only in June or July. Third-stage larvae winter in stagnant, unfrozen ponds. In some areas, characteristic fluctuations in the number of *An. pulcherrimus* from year to year have been observed, depending on the water level in the rivers of the plains, the presence of flood-lands, and water supplies for irrigation purposes. In years with low water levels, flood-land ponds dry up and the numbers of the species decrease. In rice cultivating districts, the numbers of this species are stable, except for during extremely arid summers, when, due to water shortages, rice-planting areas are considerably decreased. It is in these very areas that *An. pulcherrimus* is considered the most significant malaria vector.

An. claviger

An. claviger is an exophilic species with psychophilic larvae. The species is widely distributed. Its northern border lies slightly to the south of that of *An. maculipennis*. The southern border is characterized by an average annual temperature of approximately 20°C. The species is found in springs, wells, and other cool or cold water sources with an optimal temperature of 14-16° C, but within 7-21°C. In the north, this species is found in the plains, while in the south, it favors mountainous regions. Only very rarely may *An. claviger* serve as a malaria vector, and only if certain favorable conditions are present.

The characteristic biological feature of this species is the psychrophility of their larvae. *An. claviger* larvae reside in spring ponds supplied by sub-soil waters. Their period of development extends from 18–20 to 32–33 days. *An. claviger* larvae can tolerate shading and the absence of aquatic vegetation. A low percent of water oxygenation is characteristic for breeding sites of *An. claviger*.

An. claviger are found in abundant numbers in the mountain and foothill areas, and the breeding of the larvae occurs in subsoil waters, springs, on slopes of gullies, by sides of river flood-lands, in mountain foothills, debris cone zones, or other areas where sub-soil waters, waterlogged areas or slow-flowing, vegetated springs are formed. Larvae at the III–IV period of age winter in the same ponds where they developed over the summer. Wintering larvae are nearly immobile and frequently remain at the pond's bottom. When their ponds warm up in the spring, diapause ends and the larvae pupate.

An. claviger is a hydrophilous species. As day resting sites, it prefers humid and cool places, mostly in vegetation.

An. claviger differs from *An. maculipennis* in its method of seeking a host: it watches for victims near its breeding sites, never flying far from them. Its hunting habits and low mobility considered, *An. claviger* is not typically strongly linked to man; thus, its role in malaria transmission is not prominent.

The peak activity of *An. claviger* occurs in the evening, night and early morning hours at 10–26°C, more often at 13–19°C, and with a relative humidity of 51–95%. In dense vegetation, it also attacks in the daytime, and prefers to attack its victim in the open. The mosquitoes, after having sucked blood in cattle-sheds and houses, leave at sunrise and head to natural shelters where digestion of blood and maturation of ovaries occurs. Thus, *An. claviger* typifies an exophilic species. The end of the diapause and appearance of the first pupae in spring occurs in the south of its habitat in the beginning of February–March, and in the north, from early to mid-May. In northern districts, there is one summer generation and one wintering one. In the south, winged mosquitoes are found over a five-month period. Intense reproduction occurs in spring and autumn. The first peak in numbers occurs in May–June; during hot months, numbers fall again, starting to rise only by September–November. This mode of dynamics in the numbers of the species is explained by peculiarities in temperature at their breeding sites: in summers, breeding occurs only at the coolest sites, because a significant part of spring ponds at the height of summer either dry up or become overheated. The drastic decrease of the specie's numbers in summer, the period favorable for malaria transmission, diminishes its role as a malaria vector.

In mountain regions, where precipitation and snowfall are common and springs are less heated in summers, the decrease in numbers in high summer is less pronounced.

In vitro, *An. claviger* have been well infected by three types of human malaria (*P. vivax*, *P. falciparum*, *P. malariae*). In the Russian European Region and in Siberias this species is sparsely distributed and has no epidemiological significance. Nevertheless, in the Caucasian foothills and the mountain areas of Middle Asia, where *An. claviger* is sometimes numerous and breeds near settlements, it may become a malaria vector (as in, for example, the Adler district in Palestine).

An. plumbeus

An. plumbeus is a mosquito which dwells in the woods and breeds in the hollows of trees. Breeding may also take place in wells, barrels and other spaces with firm vertical walls. The larvae and eggs of the species can survive the winters. While the species is exophilic, it may also be endophagous. Numbers of this species tend to increase in rainy years, when it may become a malaria vector.

The northern border distribution of *An. plumbeus* Theobald passes through Rostov-on-Don, Krasnodar, Pyatigorsk, and farther down along the Terek river. In the Caucasus, this species is found in the mountains. Through humid districts of Lenkoran in Azerbaijan and northern Iran, it reaches the forested mountain gorges of Turkmenistan (Kopet-Dag). It is also found in the forested mountains of southern Tajikistan (Keshian, 1941).

An. plumbeus represents a single species which breeds in the hollows of beech, ash, hornbeam, and other types of trees. It populates deciduous forests of humid and moderately warm climates, where hollow ponds can be preserved for long periods of time. The water temperature in the hollows must be stable and quite low (not higher than 20° C) in comparison with air temperature and that of open ponds. The duration of development of larvae at 20° C is 25 days. Development may also take place at 8–12°C, but occurs very slowly. Larvae demonstrate a highly negative phototaxis and take cover in the darkness. Should the hollows dry up, the larvae can remain within the humid silt found at their bottoms for several weeks, and eggs can survive for several months. When the hollows dry up, *An. plumbeus* females lay their eggs in wells, indentations in rocky spring beds, tanks and other artificial containers with hard walls, although they avoid laying in ground-walled ponds.

All stages of larvae winter; and wherever the hollows freeze through, the eggs may winter as well. If hollow water freezes for a short time, some of the larvae survive. Extended periods of freezing temperatures within the hollows hinder the species' advancement to the north.

The *An. plumbeus* imago are hydrophilous and poorly sustain dryness of air. As day resting sites, they prefer tree hollows, cavities in rocks, slopes of gullies, cemented wells and pits, and dense vegetation. The mosquitoes attack hosts both indoors and outside. Following a blood meal, they fly out in the open, where digestion is completed. Thus, *An. plumbeus* is an exophilic species.

Just as *An. maculipennis*, *An. plumbeus* may concentrate at sites where hosts tend to accumulate: its day resting sites in nature are usually located on the outskirts of settlements or near forest paths frequented by man. In the depths of the forest, far from animals and man, the number of *An. plumbeus* is significantly lower. Within the northern reaches of their habitat, the wintered larvae grow wings at the end of April or beginning of May. Fly-out of the first generation of these mosquitoes generally occurs at the beginning of June.

The mode of dynamics of winged *An. plumbeus* numbers strongly depends upon the amount of rainfall which occurred preceding the winging process. Large numbers of *An. plumbeus* are recorded only in rainy summers, when the species may become a malaria vector in some settings of the Black Sea coastal resorts, areas in which there are no cattle to distract the mosquitoes from man. This is confirmed by the presence of

malaria parasite-inoculated mosquitoes and the results of precipitation reaction results showing a large number of females (over 10%) that have been feeding on human blood. However, the *An. plumbeus* numbers are high only in rainy years, and only then can it play a significant role in malaria transmission.

An. algeriensis

An. algeriensis is a rare species that populates waterlogged areas of Europe, Caucasia and Middle Asia. In the north of Western Europe, its habitat stretches up to England. It is found on the western coast of Estonia (Saaremaa island) and is distributed in the Trans-Caucasian Region, Northern Caucasus and in Middle Asia.

This species, as a rule, is relatively scarce. Appearance of the species in mass has been observed in river fluxes, for example, that of the Sulak river (Yenikolopov) in Dagestan. The specie winters in a larval phase, and is not a malaria vector.

Larvae are found both in large, waterlogged areas, usually densely vegetated (often by water pines), and in small but highly shaded ponds, mainly in stagnant and often highly saline water. In Tajikistan (Glagoleva 1944), larvae are well adapted to waterlogged areas overflowing with brackish alkaline water and are often found side by side with *An. hyrcanus*. The larvae winter. Mosquitoes attack man and animals in the open. In some Middle Asian areas, particularly Tajikistan, they are most typically found during the colder seasons of spring and autumn. Imago tend to inhabit reed bushes, occasionally flying into cattle-sheds and, even more rarely, into living quarters.

An. labranchiae

An. labranchiae is a Western Mediterranean species, and in these areas it has often served as a primary vector. It is distributed in the east as well, stretching into Yugoslavia and Libya. In the north, its habitat stops below the Pyrenees and the Alps. The species was never detected within the borders of the former USSR.

Mating occurs in swarms. The females winter in warm premises and feed on blood in winters.

The Anopheles mosquitoes – malaria vectors and their epidemiological significance in countries of the WHO European Region

The Anopheles genus mosquitoes are human malaria carriers, and, as all other sanguivorous mosquitoes, represent heterotrophic insects: their eggs, larvae and pupae inhabit water, while the adult insects live in the air.

The females lay their eggs on water surfaces, either one at a time or in "rafts." Within 48–192 hours (depending on water temperature, as development takes eight days at 10–12°C, and two days at 25°C), the eggs hatch and larvae emerges. Most of the time, Anopheles larvae remain on the water's surface in a level position, usually in proximity to an object and clinging to it with the hamuli of their tail hairs. One of the conditions for mass larvae development is the presence of mooring-line substratum in the anopheline-populated ponds. The Anopheles larvae feed mainly by means of filtration of water containing food particles. At the same time, they are also capable of scraping food from the substratum and chewing on water-plants and some planktonic organisms. The

larvae feed on ultramicroscopic particles, organic detritus meals, bacteria, protozoa, and other such matter. Since the larvae are indiscriminate filtrates, it is possible to make use of insecticides sprayed onto the water's surface. Insecticides intended for consumption by first stage larvae should be powder-like and not exceed 20 microns.

The vital activity of the *Anopheles* larvae in Middle Russia occurs in stagnant or slow-flowing water ponds with temperatures from 10 to 35° C. The optimal temperature for development is 20–30° C.

Larvae development consists of four stages. The water phase development ends with the formation of pupa that is mobile but does not feed.

Within the water-based phase of development, mosquitoes tend to choose pools with calm water, an absence of surf, and abundant vegetation (*Elodeides* and planktonic *lemnides* are most suitable for the *An. maculipennis* group). They cannot survive under conditions in which water current is greater than 0,2–0,4 m/sec within moderately vegetated ponds. Drastic fluctuations in water level are also hazardous to their lives. Since any sudden change of water regime in anopheline ponds, including destruction of vegetation, increase in velocity of current, water level fluctuations, and other events lead to death of the larvae, the elimination of vegetation and changes in water regime brought on by artificial means are used for mosquito control. The routine drying up of the check rows helps to prevent the *Anopheles* larvae from breeding in rice paddies. Another method for control is that of artificially shading the ponds, since the majority of the *Anopheles* fauna in the European Region develop within well-illuminated sites (except *An. hyrcanus*, *An. claviger* and *An. plumbeus*).

After emerging from the pupae, the adult *Anopheles* mosquitoes dwell for some time in the surrounding vegetation. If endophilic, they will subsequently fly out into premises and settle themselves in shaded, wind-less and draught-less places on ceilings or upper parts of household furnishings. Due to their negative geotaxis, they will always remain in a head-up position. They rest at an angle to substratum, with the body rectilinear, usually with rear legs hanging freely. Only the wintering *Anopheles* females press their abdomens to walls, and during this time, it is difficult to distinguish them by the resting posture from non-malarial mosquitoes.

After leaving the pupae, exophilic mosquitoes accumulate outdoors and choose as their day resting sites thick grass near the ground's surface in which shaded and wind-free spaces are formed and the daytime temperature is lower and humidity higher than in the open. Day resting sites of the exophilic *Anopheles* species are also comprised of tree hollows, holes, gaps in the ground, and similar spots.

During the first days of life, the un-stretched stomach of the female contains a greenish meconium mass. As it is extracted, the female's body colour becomes fulvous. When the folliculi in their ovaries reach the II stage according to Christopher's, the females start hunting for hosts and feeding on blood (at times when their development is supported by the larvae's adipose deposits, they may feed on sweetening agents, or sometimes on blood, as in the case of *An. superpictus*).

In the daytime, the males remain in the same shelters as the females. They feed only on sweetening agents, and do not feed on blood. The majority of the *Anopheles* species mate in the open air. Sometimes the *An. sacharovi* males swarm indoors, at which time

mating may occur indoors too. As a rule, *An. atroparvus* and *An. superpictus* mate indoors, although this is known to happen in the open as well.

After fecundation and the primary ripening of ovaries, the Anopheles female proceeds to a routine gonotrophic life, and the first gonotrophic cycle starts. Its first stage includes the hunt for host and attack. The hunger for blood makes mosquitoes excited; they develop positive phototaxis to faint light, and with the beginning of twilight, they will fly out of shelter in search of hosts.

A number of the Anopheles species make comparatively long flights towards large gatherings of hosts. These species feed primarily on large gregarious animals. Thus, the centers that attract the hunting Anopheles females are also often those places of overnight stay for men and animals. The space surrounding the center of attraction is considered its gravity area. The data on gravity areas for each settlement has great practical significance for the planning and carrying out of Anopheles larvae control activities.

In areas where settlements are in close proximity to one another, the gravity areas of separate settlements may partially coincide. Centres of attraction with overlapping gravity areas are known as conjugate centres. The permanent exchange of mosquitoes takes place between them, leading to a malarial parasite exchange as well, provided the settlements are malaria foci. The determination of conjugate centres is necessary both for a better understanding of the epidemic situation and for the determination of an appropriate means of carrying out malaria control activities, for example, the control of adult mosquitoes.

Environmental conditions, the relative numbers of different species of hosts, accessibility and location in regards to breeding sites and daytime resting sites, along with other factors, all have a profound effect on the choice of host of the Anopheles females. Attacks take place both indoors (endophagia) and outside (exophagia).

The second phase of a gonotrophic cycle, that of blood digestion, occurs indoors among the endophilic mosquitoes and outside among those which are exophilic. Duration of the cycle depends on air temperature and relative humidity. As it had been established by M.F.Shlenova (1938), the duration of blood digestion of *An. messeae* at the relative humidity of 70–80% fluctuates from 548 (at 7,5°C) to 41 hours (at 30°C).

The blood-fed females in shelters lacking exogenous irritants such as smoke, bright light, and unfavorable temperature and humidity, behave in a calm manner. Mobility and positive phototaxis return only when digestion has been completed in females with mature eggs. In premises offering unfavorable settings, mosquito "rotation" occurs; that is, they pass in and out of the premises at different stages of digestion. Females with ripe eggs fly out of the premises to deposit eggs. Should they find suitable ponds, the eggs will be laid during the first night. Others return to their premises by morning, the eggs unladen. If there are many suitable ponds around mosquito shelters, the placement of ovipositions is very characteristic: the nearest ponds contain the majority of eggs, while more distant ones will contain a much smaller number.

The ovipositions farthest away in distance from the center of attraction are located at approximately 3 km, and even farther when egg-laying females are great in number. The scattering of laying females is of great importance for the species, as this ensures

the more even use of ponds by mosquitoes. In ponds with a high number of ovipositions, overpopulation and a high mortality of larvae may be observed. The scattering radius of laying females defines the radius of treatment of anophelogenous ponds, surrounding malaria foci, and the so-called protective belt. Such belts should be kept in mind when the spatial pattern of malaria foci is considered.

The epidemiological significance of *Anopheles* as malaria vectors is defined by a range of factors, the most important among them including 1) the rate of *Anopheles* species susceptibility to infection by human plasmodia; 2) the duration of sporozoites' preservation in salivary glands of the mosquitoes; 3) the species numbers; 4) the degree of relationship with man; 5) the individual life span of the mosquito.

The mosquito life span and age structure of the population are of utmost importance. With each blood meal, the chances of infection of the *Anopheles* female and transmission of parasites to a healthy person increases. More often than not, the infected individuals are found among old females, but their ratio within the overall population is small. The larger this ratio within a vector's population, the more dangerous this population becomes in an epidemiological respect. The success of imago vector control is obtained by a decrease in number of old females within the population and by an increase of rates of mosquitoes that have never laid eggs.

Within a certain time of the season, the newly winged *Anopheles* females fall into a state of diapause manifested by inhibition of ovarian development. In the Moscow area, the first diapausing individuals appear in the end of July, and by mid-September, the diapause covers virtually all of the *Anopheles* population. The diapausing females choose cool premises, and may often rest in natural shelters for long periods of time. They generally feed on sweet plant juices, and will gradually become fat. Some species (for example, *An. sacharovi* and *An. maculipennis*) feed on blood for the accumulation of fat. This event may extend the parasite transmission period by this species in malaria foci. Moreover, these species, along with *An. atroparvus* and *An. maculipennis*, may feed on blood while wintering, which allows them to form so called "indoor" malaria foci during the cold period of year.

4.5 Determination of the physiological age of mosquitoes (Diptera: Culicidae)

M. Sokolova

One of the essential factors of the epidemiological status of the malaria vectors is the physiological age of *Anopheles* mosquito females. The physiological age is an indirect indication of the possible infectivity of *Plasmodium* in the salivary glands of a female, in the case in which it has taken in *Plasmodium* gametocytes during one of its previous blood feedings. *Plasmodium* develops in a female mosquito to an invasive stage within 2 – 3 or more gonotrophic cycles, depending on the ambient temperature. Usually the relation between temperature and the *Plasmodium* developmental period (in terms of days) in a certain region is known. That is why it is not difficult to predict the limits of the malarial season, comparing the female physiological age and the developmental period of *Plasmodium* in certain ambient temperatures. Furthermore, the physiological age of females is a direct indicator of the effectiveness of mosquito control measures. Larvicide applications should result in the senescence of mosquito populations, while adulticides may result in quite the contrary.

The physiological age of a mosquito female is determined as a number of gonotrophic cycles. The gonotrophic cycle is the period in a female life which begins with the quest for a host, continues with the process of blood-feeding, and finishes with oviposition into a water reservoir. As the number of gonotrophic cycles which the female has undergone increases, the greater its epidemiological importance.

The physiological age or the number of gonotrophic cycles is determined by the number of ovipositions. The number of ovipositions is determined by the morphological changes of ovarioles (egg tubes) in female ovaries.

There are two main processes that can take place in an ovariole during a gonotrophic cycle:

- Normal oogenesis, egg development in a follicle (egg chamber) and ovulation, with granulation zones and a specific basal plate formation,
- Abortive oogenesis, follicle degeneration with "dilation" forming as a result

Using the intra-ovarial neutral paraffin oil injection and neutral red staining, it is possible to open the ovaries and analyze the intact ovarioles.

Principles of mosquito age – grading

- Examination of several ovariole variations in ovaries of a certain female and attempts to make schematic drawings of them.
- Comparison of the set of ovariole variations and the developmental scheme (the number of gonotrophic cycles).
- Determination of the number of ovulations, which is equal to number of gonotrophic cycles and the physiological age of a female.

Should it be useful to define nulliparous/parous age groups, more approximate approaches, such as tracheolar and basal plate methods, based on changes in the tracheolar system (uncoiled/coiled) and ovariole basement changes (neutral red uncoloured/coloured), may be applied.

4.6 Biological control of malaria vectors in Kazakhstan: Operational experience

M.Baijanov

Field trials for 15 Bti-based preparations were carried out in the southeast of Kazakhstan. All 15 proved to be safe for concomitant aquatic life of the mosquito larvae. Only by exceeding treatment dosage by 100 times were 23-30% of chironomides eliminated. The most susceptible mosquitoes turned out to be those of the species of *Aedes*, *Culex* and *Uranotaenia* genera, while *Anopheles* proved only moderately susceptible, and *Culiseta* showed very low susceptibility.

Action depended on a range of abiotic environmental factors, water pH, and, particularly, to temperature. In natural settings, the bacterial preparations were most

effective at 30-31°C. In colder water, their action lingered: at 20-25°C it became apparent in 2 days time, and at 15°C, only after three days.

A few forms of Bactoculicide and Turidipter underwent trials. The former was most effective in pure water, and to obtain same effect in contaminated water, twice the dosage was needed. Specific characteristics of the water sources affected the action as well. In open ponds, low dosages proved effective, whereas under vegetated conditions, they needed to be increased by 2-4 times.

At present, three forms of bacterial preparations exist: liquid, granulated and powdered, applied depending on local conditions. The combined use of larvivorous fish and bacterial preparations is considered highly effective. The price of treatment for one hectare with liquid Bti approximates US \$ 12, while the cost of powder is around US \$8.

The search for new natural pathogens continues.

4.7 Prospects of bacterial agent application in the control of larval stages of malaria mosquitoes

E. Sokolova, N. Kulieva

The 14th serotype of *Bacillus thuringiensis* (Bti - strain ONR-60A) differs from other serotypes in its absence of thermostable beta-endotoxin, which may cause undesirable body alterations in haematothermals. This bacterium synthesizes delta-endotoxin in the form of oval-shaped crystals that selectively affect the larvae of Diptera (mosquitoes, midges). We began development of "Bactolarvicid" in 1979, created on the base of a domestic natural strain. The compounds in the bowels of larvae are decomposed and absorbed at pH of 7-8,5 within 20-30 minutes, with death following in 15-20 minutes. "Bactolarvicid" underwent trials in many regions of the former USSR and in Vietnam. In powder form at a dosage of 0,15 g/sq.m, it causes 100% elimination of the larvae of I-IV ages within 24 hours after treatment. By the 15-20th day, its effectiveness decreases to 30-70%. At 0,05 g/sq.m, the 99-100% effectiveness is preserved for 3 days, by the 15th day after treatment decreasing to 40-60%. The same activity pattern is observed in trials of the liquid paste form of "Bactolarvicid".

The development of "Larviol" began at the end of the 1980's. It was based on asporogenous graining Bti strain, which produced a high activity endotoxin. It was revealed that a vegetative cell could synthesize from 2 to 5 endotoxin crystals. Other serovariant strains usually create just one crystal. "Larviol" does not contain spores; it is environmentally appropriate and may be used in any pond. The activity and effectiveness of "Larviol" are at the same level as that of "Bactolarvicid".

The recommended application dosages of "Bactolarvicid" and "Larviol" are 0,2-0,4 g/sq.m for paste form and 0,05-0,15 g/sq.m for powder.

4.8 Prospects of untraditional biological methods of mosquito control

O. Lopatin

A survey of various genetic control methods and growth insect regulators has been carried out. Following the sterilization of flies by radiation and their release into the wild, a decrease in population numbers was registered. Partial sterilization combined with low fertility and prevalence of males in their offspring has also been observed.

The introduction of relatively lethal genes into the population leads to the appearance of viable but temperature-sensitive individuals. Thus, at 20-25° C, the individuals will develop normally, but die at higher temperatures. They also lack a diapause.

Genetic engineering methods are carried out in attempts to transform genotypes in natural mosquito populations and introduce malarial plasmodium-resistant genes to discontinue sporogony.

To eliminate features hazardous to man, attempts to use cytoplasmic incompatibility have been made.

The above methods may be combined with the application of contact insecticides. All these methods are targeted at maintaining of a low level of population numbers rather than suppression of population. Their advantages include species-specificity, environmental safety, influence on following generations of vectors, and availability of their application when other methods cannot be used. The disadvantages relate to their high cost, the need for highly qualified staff mastering high technologies, and large-scale scientific research. Use of these methods requires the precise study of mosquitoes, including DNA analysis and fermentogram charts. All this calls for costly equipment and the deployment of re-agents.

Growth insect regulators are extensively used in Kazakhstan for locust control (i.e. Dimilin-based insecticide, which was also tested for mosquitoes). The treated individuals have not developed chitin. At a dosage of 0,05 l/hectare, a drastic decrease in numbers has been observed. While concomitant aquatic life in general is not affected, some influence on water predators and a decrease in Rotifera and Daphnia numbers have been recorded. The Cyclops exhibited no reaction to the compound. In contaminated water, the regulator's effectiveness was drastically decreased. The compound cannot be used in fish-breeding ponds.

Bti and Dimilin applied in combination has also proven effective; jointly, they produce a rapid and long-lasting action.

4.9 Significance of malaria mosquito irritability to insecticides

M. Artemiev

In practice, mosquito irritability to residual action insecticides concerns their avoidance of indoor treated surfaces. Following the complete treatment of premises, mosquitoes tend to vacate it before they acquire a lethal dose, and they search for other day resting sites, either in untreated houses or outdoors. Thus, the impact of treatment is either close to none or even harmful.

Unfortunately, very low attention is paid to irritability in comparison to that which is addressed to resistance, although sometimes the former is much more important. The following examples should be taken under consideration.

Azerbaijan

In 1974, sprayings of cattle-sheds with one insecticide and human premises with another were carried out in malaria endemic districts. Within 2-3 days time, the insecticide had completely evaporated from the household surfaces, leaving the premises virtually untreated. In cattle-sheds, however, the insecticide remained active for 1-1.5 months, and mosquitoes (*An. sacharovi*) demonstrated a high irritability to it. As a result, all female mosquitoes previously living in cattle-sheds had moved to bedrooms and other premises, and man-vector contact had thus increased. This, on its turn, led to the outbreak of an epidemic in Azerbaijan.

Afghanistan

In rice-growing districts of northeast Afghanistan, the malaria vectors are represented by the semi-exophilic *An. pulcherrimus* and the completely exophilic *An. hyrcanus*. Over a number of years, indoor spraying with DDT was carried out. The exophilic *An. hyrcanus* had more than 90% resistance to this insecticide, because it had been used in agriculture (mainly across the Soviet border). *An. pulcherrimus* resistance for many years remained at about 10%, but this species had a highly irritability to DDT. As a result, almost all mosquitoes moved to natural day resting sites. The mosquito numbers and survival to potentially hazardous age were not affected. During one season, the number of attacks on man by potentially hazardous females of both species within the treated settlement was equal to that of a settlement that had gone untreated. Therefore, spraying with DDT in such settings proved to be a waste of both labor and funds. After the indoor residual sprayings were cancelled in 1973, the funds were directed to *gambusia* cultivation and improvement of case detection and treatment. Consequently, the incidence drastically decreased.

Tajikistan

In the south of Tajikistan, as in the northeast of Afghanistan, the main vector is *An. pulcherrimus*, with *An. hyrcanus* playing a role in malaria transmission as well. Both species have single super-populations, covering areas of these two countries to the north and south of the borderline rivers Pianj and Amu-Daria. Therefore, both species' resistance and irritability in the south of Tajikistan is equal to that of northeast Afghanistan. In 1984, intensive sprayings of settlements with Malathion were initiated in the Pianj district of Tajikistan. In one season, two rounds of spraying had been carried out; at that, owing to the significant exophily of *An. pulcherrimus*, not only internal, but also external surfaces were treated. That year, just 5,9% of moderately-irritable individuals in *An. pulcherrimus* population were revealed. Mosquito numbers had drastically decreased.

By 1985, it was evident that selection of irritable individuals had occurred, and the share of moderately-irritable reached 20%, to which some 6,7% of highly-irritable were added.

In 1986, just one round of indoor residual spraying was conducted, and the pressure of insecticide was almost taken away. Nevertheless, due to the selection that had taken place in previous years, the share of irritable individuals already constituted 57,1%.

In 1987, when the sprayings were nearly discontinued, the share of irritable individuals had again decreased to levels seen initially (15,4%). A similar picture was observed in the neighbouring Parhar district, where supervision had been carried out from 1985 to 1987.

It was thus proven that mosquito irritability to insecticides used rises correspondingly according to an increased intensity in anti-imago sprayings and falls with the discontinuation or decrease in sprayings. On the contrary, resistance appears mainly in the plains, where insecticides are used for agricultural purposes. Similarly, also within Tajikistan, the highest resistance to DDT of the mountain *An. superpictus* was registered in Kuliab, which is surrounded by fields (Artemiev et al. 1987).

Laboratory trials

Trials on insectarium mosquitoes of the Martsinovskiy Institute of Medical Parasitology and Tropical Diseases have revealed that nearly all species under trial had varying irritability to the insecticides of one group - malathion and fenitrothion. This has allowed researchers to make assumptions on the irritability mechanism (Sorokin et al. 1991).

In Karakalpakia, a seasonal variation in irritability in *An. martinius* was discovered. Irritability among the diapausing females proved much higher than in summer individuals. The same fluctuations were then observed in Moscow, in a laboratory culture of *An. martinius* that had developed without diapause, although considerable time deviations were noted.

Later, N.Sorokin, through selection of males and females, succeeded in raising two *An. stephensi* lines – one completely irritable to malathion and the other completely un-irritable.

Thus, when resistance and irritability are compared, the common trait of these phenomena is their inheritability; apart from that, there are many differences.

As a rule, before a new insecticide is applied, there are no individuals that are resistant to it, whereas those irritable to it may occur. Resistance usually appears in the plains areas where agriculture is carried out extensively and pest control treatments are carried out. Indoor residual sprayings take on the role of selective factors rather late.

When irritability is present, selection occurs quickly and the share of irritable individuals grows with the increasing intensity of sprayings.

After an insecticide's action has ceased, resistance vanishes slowly. In the Parhar district of Tajikistan at the end of the 1980's, *An. hyrcanus* retained 58,3% of DDT-resistant individuals, even though this insecticide had not been used since the beginning of the decade, even in agriculture. Resistance cannot appear in areas where a given insecticide was not applied in agriculture; thus, its occurrence is hardly expected at the

end of the 1990's and beginning of the 21st century, where, due to economic and political reasons, there was almost no agricultural use of insecticides.

On the contrary, irritability quickly appears with insecticide spraying, and vanishes just as fast upon its cessation. Therefore, it may be controlled with an annual rotation of 2-3 insecticides.

5. Conclusions

The meeting held 3–5 May 2001 in Almaty, Kazakhstan provided a forum for the exchange of information on issues related to medical entomology and the application of existing methods and technologies for malaria vector control in the WHO European Region countries confronted with the resurgence of malaria.

The specific objectives of the Meeting included:

- Review of current status of the knowledge in the area of taxonomy, biology, ecology and genetics of malaria vectors in the countries of the European Region,
- Analysis of the epidemiological role and geographic distribution of malaria vectors within the WHO European Region,
- Analysis of current data on vector resistance to insecticides,
- Review of existing methods and technologies for vector control and their applicability, taking into account different eco-epidemiological and socio-economic settings within the WHO European Region.

Participants included delegations from Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Russian Federation, Tajikistan, Turkey, Turkmenistan and Uzbekistan; speakers from research institutions of the Russian Federation, Turkmenistan and Kazakhstan, WHO staff members from EURO and Kazakhstan, and representatives of the Centre of Biological Control from Israel.

Following the presentations on the part of Member Countries, reports and in-depth discussions, it was noted in conclusion that this meeting had in a sense served as the first scientific and practical forum within the last decade for the countries confronting the resurgence of malaria. The meeting allowed for the exchange of operational information on malaria and its control between Member Countries, with a special emphasis on the entomological situation and vector control interventions. Reports by well-known experts in the area of medical entomology and genetics contributed to the expansion of participants' outlooks on issues concerning the taxonomy, biology, ecology and genetics of malaria vectors, as well as the role and significance of vector control interventions in the European Region.

6. Recommendations

It was recommended for the World Health Organization:

- To continue assisting national malaria control programmes in planning, implementation and evaluation of vector control interventions,
- To continue financial and advisory support of entomological training at national levels,
- To continue advisory and financial assistance of operational entomological research, including monitoring of malaria vectors resistance/irritability to the insecticides employed,
- To continue assisting in preparation of technical reports, guidelines and manuals related to medical entomology and vector control.

It was recommended for WHO and Member States:

- To continue the taxonomic studies of the *Anopheles* species aimed at the determination of the taxonomic status of unclear forms, detection of new local species, and establishment of borders of the habitats of existing species,
- To continue studying the morphological differences of members of *An. maculipennis* complex,
- To continue studies on life cycle and ecology of main vectors, in particular the frequency of repeated feedings on man during a gonotrophic cycle, antropophilism, and ecological preferences of larvae,
- To apply immunological methods of determination of malarial mosquito infectivity by human plasmodia,
- To carry out regular assessment of local malaria vectors resistance/irritability to insecticides used both in vector control and within the agricultural sector,
- To carry out trials of larger numbers of spraying equipment models in order to determine the most suitable equipment for a particular purpose,
- To carry out study trials on new insecticides with residual action within various eco-epidemiological and socio-economic settings of the region,
- To carry out trials on larger numbers of local larvivorous and phytophagous fish in different eco-epidemiological settings of the region,
- To continue the search for effective larvivorous fish and other water predators, in particular *Mesocyclops*,
- To carry out trials on new forms of bacterial agents in order to determine which ones are most cost-effective,

- To continue research on the development of long-lasting forms of bacterial agents and the search for new entomological pathogens,
- To continue studies on the applicability of untraditional methods for biological control (growth insect regulators, sterilization, genetic methods, etc.).

It was recommended for Member Countries:

- The application of insecticides with residual action against endophilic vectors (imago) remains one of the principal malaria control options (especially within the context of on-going epidemics) in countries of the European Region. Decisions on the use of certain insecticides should be based upon an epidemiological and entomological assessment of the local malaria situation. To prevent the development of malaria vector irritability, it is essential to rotate two or three insecticides,
- To initiate at the beginning of transmission season campaigns for the distribution of larvivorous fish (gambusia or local larvivorous species) to water reservoirs, including rice paddies. For weeded reservoirs, it is recommended to combine small larvivorous fish with large phytophagans whenever possible, or to use bacterial agents based on *Bacillus thuringiensis israelensis* H-14 once in 10 days,
- To combine biological control measures with impregnated mosquito nets in areas under the influence of exophilic or semi-exophilic malaria vectors.

It was recommended for WHO and RBM partners:

- To continue financial assistance for malaria-affected countries, with priority given to those facing ongoing malaria epidemics/outbreaks, to allow for the procurement and delivery of insecticides and equipment/supplies for vector control.

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