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Continuing from the article by Marina Sokolova in the last issue of the Bulletin, here are a few abstracts from the workshop. References are not available but enquiries should be sent to Dr Sokolova (e-mail: msokolova@vigg.ru).

Morphological analysis of *Anopheles* larvae from Western Siberia

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As a result of cytogenetic analysis, the presence in Western Siberia of two species of malarial mosquitoes was revealed: *Anopheles beklemishevi* Stegnii & Kabanova, 1976 and *An. messeae* Falleroni, 1926. The purpose of the present work was the morphological description of the larva of *An. beklemishevi*, comparative morphological analysis of *An. beklemishevi* and *An. messeae* larvae and analysis of morphological variability of larvae of the two species.

An. beklemishevi larvae differ from those of other species of the *An. maculipennis* complex by the branching of the clypeal setae. In *An. beklemishevi* three types of internal clypeal setae (2-C) are characteristic: both simple; one simple, one ramified into two branches; both ramified in two branches. Internal clypeal setae (2-C) in *An. messeae* are ramified into 3 - 8 branches. In *An. messeae* larvae, the 10th lateral seta of the prothoracic pleural group (10-P) is ramified into 3 - 5 long branches. In *An. beklemishevi*, this seta is simple. Metathoracic seta 6 (6-M) in *An. beklemishevi* is simple, but ramified into two in *An. messeae*. The species differ in abdominal setae. Accessory dorsal setae 0 on segments IV and V (0-W, 0-V) in *An. beklemishevi* are simple and in *An. messeae* are divided into two branches.

The correlation of morphological variability and chromosomal polymorphism of the two species was investigated by one-factor variance analysis. The valid relationship between frequencies of inversion types in the chromosome 2R and morphological parameters such as length and width of head and length of antenna in *An. messeae* larva was revealed. In *An. beklemishevi* larvae with inversions XL1 and XL0 differences in head length and length of the antennas seta (1-A) were detected. It was shown on morphological characters that *An. beklemishevi* larvae are similar to the North American species *An. earlei*.

Distribution of the cytoplasmic bacteria *Wolbachia pipientis* in *Culex pipiens* in connection with origins of urban populations of these mosquitoes

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Culex pipiens, the northern common mosquito, is known in two forms, or ecotypes, *pipiens* form and *pipiens molestus* form, with sympatric distributions (some authors give them the rank of subspecies). Morphological differences are small, but biological differences are significant. The *molestus* form is an urban, hypogeal mosquito and is further characterised by autogeny (ability to mature the first egg batch without blood-feeding at the expense of larval reserves of nutrient materials, stenogamy (ability to mate in restricted space) and absence of diapause. The *pipiens* type form is anautogenous, eurygamous (mates in a swarm which requires a large space) and invariably hibernates in the wild.

Mosquitoes of the *Culex pipiens* complex are of great medical-veterinary significance as vectors of diseases of humans and animals, especially in tropical countries. These include lymphatic filariasis (wuchereriosis, brugiosis) and a number of arbovirus infections - Western equine encephalitis in USA, Japanese encephalitis in Asia, St-Louis encephalitis in Northern and Southern America and Rift Valley fever in Africa. These mosquitoes also serve as vectors of West Nile fever, recent epidemics of which have been recorded in New York (USA) and in the Volgograd area of Russia. The virus was isolated from *Cx. pipiens* in the Czech Republic (Hubalek *et al*., 1999) and in Romania (Nicolescu *et al*., 1998, 1999).

Cytoplasmic heritable endosymbiotic bacteria of the genus *Wolbachia*, (close to *Rickettsia*), are found in the reproductive tissues of many arthropods. The bacteria are the cause of cytoplasmic incompatibility in populations of *Culex, Aedes, Drosophila, Manduca* and *Tribolium* (Werren, 1997; Zakharoy *et al*., 2000). *Wolbachia* can be considered as a potential agent for biological control of pest species.

For the first time, using molecular-genetic methods, we have investigated *Wolbachia* distribution in four natural populations of *Cx. pipiens* from Russia. DNA isolated from larvae and pupae was amplified using primers specific to DNA 3' ends of a gene cytochrome oxydase 1 (mitochondrial DNA gene) of *Drosophila yakuba* (Juan *et al*., 1996). For detection of *Wolbachia* in total DNA we have used primers specific to a gene wsp of *W. pipientis* (Braig *et al*., 1998). DNA of *W. pipientis* was detected in three urban (hypogeal) populations from St. Petersburg (in 49 insects from a sample of 50 insects) from Moscow (in 47 of 50 insects), and Volga-Volgograd (in 15 of 17 insects). Field (natural) populations in an area 69 km from Leningrad appeared to be uncontaminated (50 insects). Thus, *Wolbachia* infections differed significantly between the populations studied, and cytoplasmic incompatibility makes gene exchange between them impossible and promotes their isolation. Examination of nucleotide DNA sequence alignment of the *pipiens* type and *molestus* forms have revealed 5 nucleotide replacements among 226 nucleotides, whereas DNA of form *molestus* from St. Petersburg and Volga appear to be identical. The incompatibility, induced by the presence of *Wolbachia,* is probably one of the causes of isolation between sympatric populations of the two forms.

Effects of males on female mosquitoes

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Because it is only the female mosquito that feeds on blood and therefore can transmit parasites, male mosquitoes tend to be ignored. However, males are important. They not only significantly affect the behaviour of the females with which they mate, but will undoubtedly be the vehicles distributing new genes to field populations of females if population replacement altering the vectorial capacity of a species ever becomes a reality. One common assumption about mosquito mating systems is that male accessory gland substances transferred to the female during mating terminate her subsequent mating behaviour for life, but this is not the case for *Anopheles gambiae*. In this species, male accessory glands have no effect on subsequent mating by the female, but mating does appear to be regulated by some other mechanism. Our preliminary experiments suggest that sperm within the spermatheca may modulate the tendency of the female to re-mate. An unusual sperm polymorphism has also been identified that may be related to female mating behaviour or be the subject of female selection. We have also demonstrated changes in cuticular hydrocarbons of female *An. gambiae* after they have mated that may provide a signal to the male about the female's mating status.

Epidemiological features of malaria in the Yoronezh area

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The malaria situation in the territory of the Russian Federation has worsened since 1996 when the introduction of malaria from southern countries of CIS began and nine secondary cases of *vivax* malaria were detected. In subsequent years the number of cases due to local transmission increased (Baranova, 2002).

The area of pools suitable for anopheline larval development in the region is about 870 ha. The dominant species of mosquito is the endophilic *Anopheles messeae* Falleroni. Mosquito population densities on average are 9.8-12.2 (imago) per 20 sq. m. of wall space with an average of 33.5% of houses infested. An average of 33.6% of available pools is actually positive for mosquito larvae.

The highest number of imported cases of malaria in the Voronezh area was recorded in 1995 (13 cases) and in 1997 (16 cases). In total, 21 malaria cases, including 7 children up to 14 years old, were detected in the area during the period 1999-2001, with *Plasmodium vivax* accounting for 66.6 % of cases. Most imported malaria was from Azerbaijan and Tadjikistan, but some came from the Astrakhan area. Cases coming from distant countries (Pakistan, Sudan and Sierra-Leone) accounted for 33.3% of imported malaria during the last 3 years. In 1999 one autochthonous case of transmission was registered.

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For the majority of patients (65%), clinical symptoms lasted from several days to one month after returning from the malaria foci. For the remaining patients late development of disease was characteristic. Analysis of malaria cases confirmed that the demand for medical care occurred 1-5 days after beginning of the disease in 86% of patients. In 28% of cases errors of clinical diagnostics took place. Malaria parasites were not always detected in blood preparations, therefore, treatment was not given until 10 or more days after the appearance of clinical symptoms in 9.5% of cases. This was due to the absence of qualified physicians and laboratory technicians.

A priority of malaria prevention in the Voronezh area is education of medical staff in the diagnosis and treatment of malaria.

Malarial situation in the Republic of Kazakhstan

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The epidemiological situation of malaria in Kazakhstan has become complicated since the middle 1990s, when interstate communications between countries with malaria risk was inaugurated. Since 1991 most cases of malaria registered in Kazakhstan were imported, the annual incidence being: 1991 - 2; 1992 - 8; 1993 - 9; 1994 - 24; 1995 - 4 1; 1996 - 87; 1997 - 102; 1998 - 88; 1999 - 53; 2000 - 37; 2001 - 3 1. In the first 9 months of 2002 a total of 17 cases were detected (13 - *P. vivax*, 1 - *P. malariae*, 1 - *P. vivax* + *P. malariae* and 2 - *P. falciparum*).

It was established that the introduction of tropical malaria has taken place during investigation of cases of *P. falciparum* malaria. Exit visas for a business trip to Nigeria for two persons were issued in Moscow, where vaccinations against yellow fever was made and drugs for malaria chemo-prophylaxis were offered. However the drugs were refused because of their high price.

Most imported malaria comes from Pakistan, Afghanistan, India, Turkey, Nigeria, Equatorial Guinea, Tadjikistan, Kirghizia, Azerbaijan and Uzbekistan. The annual numbers of cases detected have been: 1996 - 1 case, 1998 - 4 cases, 1999 - 1 case, 2000 - 7 cases, 2001 - 2 cases, 2002 (first 9 months) - 1 case.

Practically all of the territory of the Republic of Kazakhstan, with the exception of Mangistauskaya area, is considered to some extent as malariogenic. The malaria epidemic season lasts 1.5 - 2 months in north Kazakhstan, and 3 - 4 months in places in the central and southern zones of the Republic where *An. superpictus*, *An. martinius*, *An. atroparvus* are widespread. In Kazakhstan the majority of malaria mosquitoes are endophilic, but include two exophilic species, *An. hyrcanus* and *An claviger*.

There is an estimated 6000 ha of pooled water suitable for anopheline larvae within 3-5 km of villages. There is an additional 70 ha. of rice cultivation in three regions. The species composition of the mosquito populations and the temperature (number of days in a year with mean diurnal temperature higher than 16^{o}C), make for a high hazard of malaria in Almatinskaya, South-Kazakhstan, Zhambylskaya, Karaganda, Aimaty city; and a medium hazard in Aktyubinsk, Akrnolinsk, and a low hazard in Kostanayskaya province.

Malaria situation in the Republic of Uzbekistan and malaria preventive measures in the post-eradication period (1961-2002)

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In the pre-eradication period (1924-1946) in the Republic of Uzbekistan three forms of malaria were widespread: tertian (*P. vivax*) (75-85%), tropical (*P. falciparum*) (13-21%) and quartan (*P. malariae*) malaria (2-4%). The morbidity was between 254.6 and 1321.3 per ten thousand population. Following broad application of insecticides and hydro-technical measures, malaria was eradicated by 1961. For 38 years there was no malaria transmission in Uzbekistan, except for the prison in Uygursk of Papal region of the Namangan area, where malaria was imported from Afghanistan.

From 1961 to 2001, 1702 cases of malaria (tertian - 1638, tropical - 58, quartan - 4, mixed - 2) were imported. Of these 59.3% was imported to cities, 40.7% to the countryside.

Among seven species of *Anopheles*, the main vectors are the endophilic *An. superpictus* and *An. maculipennis*, and the semi-endophilic *An. pulcherrimus* and *An. martinius*. The exophilic *An. hyrcanus*, *An. claviger*, *An. algeriensis* remain secondary vectors. In contrast to past years, the amount of surface water suitable for larval development has been considerably reduced due to the progressively improved hydro-technology.

Agricultural areas under irrigation in the Republic total 4.2 million ha, and the length of the irrigation network is more than 58,000 km. During the irrigation process numerous artificial pools suitable for development of *Anopheles* larvae appear in the fields. The temperature is suitable for rapid development of vectors (up to 8 generations) and plasmodia (up to 6 cycles), and malaria transmission extends over a period of about 5 months. The peak of mosquito abundance is during August and the first half of September. At present high numbers of vectors occur in the Surkhan-Darya area on the river Surkhan, the Kashkadarinskai area on the river Tankhaz, and in the Fergana area on the river Sokh, where *An. superpictus* is found.

Anopheles control is conducted as entomological findings indicate. In 2001 13.1 million m^2 of buildings were treated with insecticides; 17.2 thousand ha of pools were treated with larvicides, and 4486 ha of pools were populated with *Gambusia*. Areas suitable for larval development were reduced by the employment of suitable sanitary-hydro-engineering measures. By the joint efforts of the Ministry of Agriculture Water Service and Municipal Services the following environmental and maintenance work was carried out: clearing of 36144.4 km of irrigation channels; filling of 235 ha of pools; draining of 3262.2 ha of waterlogged places.

Malaria return in Kyrgyzstan

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Up to 1998 mainly imported cases of malaria were registered in the Republic. However, in this and subsequent years local transmission of tertian malaria appeared: in 1998 there were 5 locally transmitted cases in a total of 13 malaria cases, in 2000 there were 7 of 12, and in 2001 - 1 5 of 28. In 2002 the disease occurred in epidemic proportions, with 2267 cases, mostly in the Osh area. The main reasons for this return of malaria are:

- Continuing epidemics in Tadjikistan.
- Intensive human population migration.
- Extension of the areas of rice plantations.
- Increase of the amount of surface water suitable for anopheline larvae.

To these must be added pre-existing problems of.

- A deficit of qualified personnel.
- Lack of insecticides and equipment for control of malaria vectors.

Epidemiology of malaria in Georgia

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In Eastern Georgia *An. maculipennis, An. claviger, An. superpictus, An. hyrcanus, An. algeriensis, An. plumbeus* and *An. sacharovi* are widespread. The main transmitter is *An. maculipennis* and the minor vector is *An. sacharovi*. The period of seasonal activity of *An. maculipennis* is March to October, with maximum numbers in August. In Western Georgia *An. maculipennis, An. claviger, An. hyrcanus, An. plumbeus* (mainly found in the forests of Abkhazia), and *An. melanoon* are widespread. The most important vectors are *An. maculipennis* and to a lesser extent, *An. hyrcanus*. The period of seasonal activity is from February to November.

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Locally transmitted malaria was not detected, from 1970 to 1996, but 139 imported cases were registered as a result of people returning from 22 Asian and African countries, most being servicemen returning from Afghanistan during the 1980s. In 1996 three cases of locally transmitted tertian malaria occurred among frontier guards in the Lagodekh region near the frontier with Azerbaijan. In 1998 there were 14 locally transmitted cases and this figure increased yearly: in 1999 there were 35 locally transmitted cases, in 2000 - 164, in 2001 - 438, in the first 8 months of 2002- 396. In 2000, one case of imported tropical (*P. falciparum*) malaria was registered. Major causes of concern are new malaria foci appearing in the Gardabansky (Quemo kartly), Lagodekh, Sagaredjsky, Sygnagsky and Dedoplitskaroysky regions of Kakhetya. These are hypersensitive foci, where malaria was formerly endemic and local transmission was stable.

Malaria vectors in the Azerbaijan Republic

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Malaria in Azerbaijan was practically eliminated by 1960. A few small epidemics were registered in the period from 1963 to 1975 and from 1979 to 1983. The situation worsened after 1993, and in 1995 there were 2840 malaria cases, in 1996 - 13135, 1997 - 9911, 1998 - 5175, 1999 - 2315, 2000 - 1526, 2001 - 1058, and in the first 9 months of 2002 - 451 cases. Ten regions and towns in the Kura-araksinskaya and Lenkoran valleys, where refugees and enforced settlers concentrated, appear to have been most vulnerable.

Seven species of malarial mosquitoes, *An. maculipennis, An. sacharovi, An. subalpinus, An. superpictus, An. hyrcanus, An. claviger* and *An. plumbeus* are found in Azerbaijan. The main vectors are *An. maculipennis*, which is widespread and is the most effective malaria vector, *An. sacharovi* (endophilic), widespread in the Kura-araksinskaya valley, and *An. subalpinus*, limited to the Lenkoran lowlands. *Anopheles plumbeus, An. hyrcanus,* and *An. claviger* are secondary vectors and are exophilic.

Ecologically sound mosquito abatement methods are employed, namely application of *Gambusia* to pools to control mosquito larvae; planting of *Eucalyptus* to dry out marshy areas; and the treatment of buildings with pyrethroids.

Entomological situation of malaria in the Republic of Armenia

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Malaria was eliminated in Armenia in 1963 but re-appeared in 1994, when 196 imported cases were registered at the frontier with Azerbaijan. In 1995 the number increased to 502 cases. In 1996 149 locally transmitted cases of tertian malaria are detected, in 1997 a total of 841 cases included 567 which were transmitted locally, predominantly in Ararat and Armavir provinces. The greatest number of cases, 1156, was in 1998 and included 542 locally transmitted. In 1999 the number of cases decreased to 616, including 338 locally transmitted cases, in 2000 to 141, including 56 locally transmitted cases. In 2001 79 malaria patients were registered, including 31 as a result of local transmission.

In Armenia the six known malaria vectors are: *An. maculipennis, An. sacharovi, An. claviger, An. hyrcanus, An. superpictus* and *An. plumbeus*. *Anopheles sacharovi* has up to 5-6 generations annually, but this species is abundant only at the beginning of summer. In the same territories, *An. superpictus* is abundant throughout the summer, with peak numbers at the end of summer. Both species are capable of supporting intensive transmission of malaria from June to October.

A highly effective insecticide, Zolvak 10%, is used for control with adult mosquitoes. In 1999 the total area of standing waters was estimated to cover a total of 2642 ha; in subsequent years this area increased (in 2001 to 4276 ha, including 740 ha where *Gambusia* was breeding). A total of 251 ha were treated with Bactoculicide in 1998-1999. About 90% of local cases of malaria are registered in Ararat Valley, where agricultural irrigation is widely practised and the area of pooling inhabited by anophelines is extensive.