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A general characterisation of the mosquito fauna (Diptera: Culicidae) in the epidemic area for West Nile virus in the south of Romania

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An epidemic of neurological infections (mainly meningoencephalitis) occurred between July 15 and October 12 1996 in the south of Romania. From a total of 767 clinical cases, 393 cases were diagnosed as acute West Nile fever infections. Roughly half of these 767 cases were registered in Bucharest and its suburbs. The lethality was about 4.3% in persons over 50 years old. Both disease incidence and fatality ratio increased with advanced age. The general incidence was 4.1 per 100,000 population (Tsai *et al.*, in press). During the same period of 1997, 14 meningoencephalitis cases produced by West Nile virus were confirmed in the same area.

The West Nile flavivirus persists in nature in the enzootic cycles involving birds and mosquitoes. Transmission to humans is tangential and dependent upon favourable weather patterns increasing vector populations and amplifying virus transmission rates. A West Nile virus strain was isolated from a *Culex pipiens* mosquito pool collected inside a block of flats in Bucharest at the beginning of October 1996. This species seemed to be the epidemic vector; it was the predominant mosquito species over the area in the epidemic period.

The data relating to the mosquito fauna in the south of Romania could be relevant in explaining some of the aspects of West Nile virus circulation within this area, because the epidemic occurred in the lower Danube valley including Dobrudja and the whole Romanian Plain, including the capital city, Bucharest. The main results of more than 20 years investigation of the mosquito populations in the south of Romania are presented.

The Romanian Plain is situated in the south of the country, between the hills which border the Carpathian mountains and Moldavian table land in the north and the Danube river with its meadow in the south and east. The plain is narrow in its western part (30-40 km) and it widens to the east (130-148 km). The height of the relief decreases both to the south and east from about 200m to 10-20 m. Dobrudja is bordered by the Danube River. Its north-eastern corner is represented by the Danube Delta. Dobrudja has a rather undulating relief of 100-200 m with lower areas on the Black Sea shore and in the Danube Delta.

Climate factors are homogenous all over this low and even territory, so that the south of Romania is characterised by a continental temperature climate with a tendency to dryness. The annual mean temperature is 10-11°C; January isotherms are minus 3-4°C and July isotherms are 22-23°C. The annual rainfall totals 500-550 mm (maximum 950-1100 mm) and even less on the Black Sea shore. They drop in an irregular manner and generally are quantitatively reduced in autumn. The dominant winds are easterly and north-easterly.

The whole Romanian Plain is crossed from the north to the south, southeast and east by the Danube tributary rivers running to the lower altitude. Lakes and ponds are scattered through the territory, often in the river valleys and especially along the Danube and on the Black Sea shore.

The vegetation is typical for the steppe but most parts of the territory are now covered by agricultural land. There are also scattered deciduous forests, remnants of the vast forests of the past, with species of *Quercus*, *Fraxinus*, *Acer*, *Ulmus*, *Tilia*, *Carpinus*, *Populus* and *Salix*.

Many rural and urban settlements are scattered over all of this territory so that the natural environment suffers a strong anthropic intervention. The mosquito fauna is rather diverse and very abundant throughout almost all this territory which provides a great variety of natural and artificial habitats (Nicolescu, 1995).

During the last 20 years, 39 mosquito species from 7 genera have been found in the south of Romania:
Genus *Anopheles*: *atroparvus*, *messeae*, *maculipennis* s.s., *claviger*, *hyrcanus*, *plumbeus*;
Genus *Aedes*: *cinereus*, *geminus*, *vexans*, *geniculatus*, *annulipes*, *cantans*, *caspius*, *dorsalis*, *duplex*, *detritus*, *excrucians*, *flavescens*, *intrudens*, *leucomelas*, *pulcritarsis*, *punctor*, *sticticus*, *refiki*;
Genus *Culex*: *modestus*, *hortensis*, *martinii*, *pipiens*, *territans*, *theileri*, *torrentium*;
Genus *Culiseta*: *annulata*, *alaskaensis*, *longiareolata*, *subochrea*;
Genus *Coquillettidia*: *richiardii*, *buxtoni*;
Genus *Orthopodomyia*: *pulcripalpis*;
Genus *Uranotaenia*: *unguiculata*.

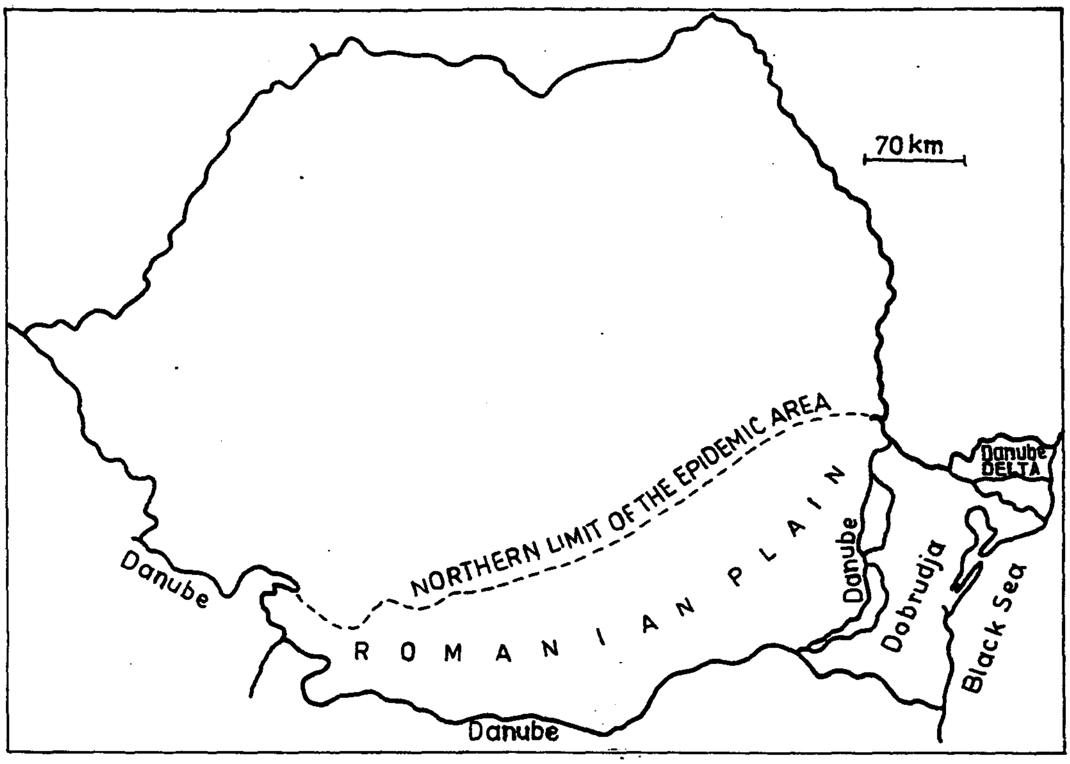

Fig. 1. The epidemic area for West Nile virus in the south of Romania in 1996.

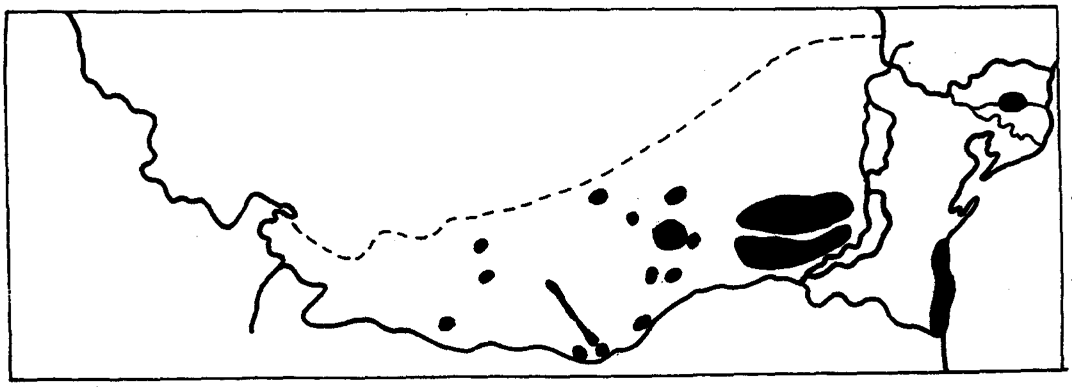

Fig. 2. The areas investigated for mosquito fauna in the south of Romania.

The natural habitats for the mosquito larvae are especially connected to the Danube and the valleys of its tributaries. The permanent or temporary breeding sites, e.g. lake edges, ponds and marshes are provided with water by floods or rainfalls in the spring or by raised hydrostatic levels. In the forests, temporary ground pools resulting from snow melt, and tree hollows are the prevailing breeding sites.

Beside the natural habitats, there are a variety of artificial breeding sites in both rural and urban settlements. In villages, these breeding sites are connected to human activities in agriculture and cattle breeding. These activities increased after 1989, when people recovered their own lands and resumed cattle breeding on a larger scale. Also, many of these activities, especially gardening and cattle breeding, moved inside households.

In urban areas, including Bucharest, the most prevalent breeding sites are connected to the flooded basements in some blocks of flats and to shortcomings of water supply, and wastewater and excreta disposal networks. On the other hand, in some urban areas there are also households with gardens for vegetables or poultry. They contain almost the same type of breeding sites as households in the villages, especially various kinds of containers to store water. Another factor influenced Bucharest especially as, in the eighties, demolition action affected about one third of the city. There are still empty areas, sites containing building in various stages of construction and foundation pits. In these areas there are numerous artificial sites for mosquito development.

All of these types of artificial habitats are particularly suitable for the development of *Culex pipiens* larvae and they yield very abundant populations of adult mosquitoes both in rural and urban environments. They find appropriate hosts and resting sites inside human dwellings or inside the households. It is obvious that many habitats both for larval and adult mosquito populations can be found together in human proximity, encouraging almost permanent human-vector contact.

We shall try to make a very general estimation of the spreading of different mosquito species in the south of Romania taking into account the presence of these species in 17 investigation areas during the last 20 years. These areas contain practically all types of natural and artificial ecosystems existing in this territory. All of these areas were investigated at least during one whole year, but half of them were successively or intermittently investigated during several years. The presence of every mosquito species in these areas can be transposed over the entire southern Romanian territory.

Thus the *Culex pipiens* species, including its form *molestus,* and the local anopheline species of the *maculipennis* complex are distributed over all this territory. *Culex pipiens* s.l. is more abundant in the urban areas where the endophilic and autogenous populations of its form *molestus* are present together with exophilic and anautogenous ones.

In rural areas, in the immediate proximity of humans, beside *Culex pipiens* s.l. there are abundant anopheline populations concentrated in animal shelters. There are usually mixed populations containing the three listed species of the *maculipennis* complex, dominated during the last years and on the greatest part of this territory by *Anopheles atroparvus*. In the Danube meadow, in the lower valleys of its tributaries, and also in the Danube Delta, the very abundant anopheline populations are dominated by *Anopheles messeae* (Nicolescu, 1996).

Each of the species *Aedes caspius*, *Aedes vexans* and *Culex modestus* is spread over more than three quarters of the southern Romanian territory while *Culiseta annulata* is present over almost two thirds of this territory, in spite of the fact that its populations are not so abundant. *Aedes caspius* and *Aedes vexans* are present generally in abundant populations especially in natural habitats and also in both rural and urban environments. *Culex modestus* is very abundant in meadows and in the Danube Delta. It is a constant component, more or less abundant, of the mosquito fauna in areas where reeded waters are present.

Aedes geniculatus, Aedes annulipes, Aedes cinereus, Aedes dorsalis, Aedes flavescens, Culex territans, Coquillettidia richiardii and *Anopheles claviger* were found merely in a third to a half of the investigated areas. *Aedes geniculatus, Aedes annulipes* and *Aedes cinereus* are constant components of the mosquito fauna in the forests, the first two species reaching high densities in some places and circumstances. *Aedes dorsalis* accompanies *Aedes caspius* in areas where the larval habitats contain saline water, but this species appears in lower densities compared with *Aedes caspius. Coquillettidia richiardii* appears in rather high densities in areas with extended stagnant reeded waters, and together with *Aedes caspius, Culex modestus* and *Anopheles hyrcanus* in the Danube Delta. *Anopheles plumbeus, Aedes leucomelas* and *Culex martinii* appeared in a quarter of the investigated areas. The remaining 20 species were found in less than 20% of the investigated areas and some of them very rarely (once or twice) and only in some very specific habitats.

There are great annual variations of the mosquito fauna due to the variation of the climatic factors, especially rainfall and temperature, and also due to human intervention in some areas. A very conclusive example is represented by the variation of the mosquito fauna in the Cernica Forest, near Bucharest, during 1986 - 1991 (Cioplan *et al.*, 1990; Nicolescu, unpublished data). Seventeen mosquito species were recorded as adults over that period in the forest, but the annual number of species varied between 6 and 14. The smallest number of species was registered in 1987, when a very hot and windy end of spring and summer caused the drying of breeding sites of *Culex* spp. and *Anopheles maculipennis* s.l. The composition of the mosquito associations and the abundance of every species also varied. The dominant species in 1987, 1989, 1990 and 1991 was *Aedes vexans* together with *Coquillettidia richiardii*, and sometimes also with *Aedes geniculatus*. *Coquillettidia richiardii* was a major component of the mosquito fauna every year because of the presence of a large permanent pond in the forest, but it dominated this fauna only in 1986 together with *Culex pipiens* and *Aedes geniculatus*, and in 1988 again with *Aedes geniculatus*. In 1987, *Coquillettidia richiardii* accompanied the dominant species, *Aedes vexans* which developed in the spring prior to the drying of the ground breeding sites in summer. In 1988, from May 3 to June 5, three chemical treatments were applied over the forest against the unusual high attack of *Tortrix viridana* and *Lymantria dispar* caterpillars. This resulted in the adult mosquito fauna being flatly dominated by *Coquillettidia richiardii* (59.4%) and *Aedes geniculatus* (26.7%) which developed in breeding sites unaffected by the treatment. Adults of the other 9 species, especially spring aedines, which occurred particularly early that year, were almost totally eliminated. There are the same general characteristics of the succession of mosquito species over all the south territory of Romania.

The appearance of larval mosquito population in the spring depends strongly upon the temperature. In March and April, but sometimes beginning even with February, *Aedes annulipes*, followed very soon by *Aedes cinereus*, *Aedes caspius*, *Aedes vexans* and other aedine species, appear in temporary habitats from snow-melt and rainfall and in flooded areas. Some of these species can be found as larvae until late May and June usually due to the gradual immersion of the soil containing aedine eggs. The adults of these species can begin to appear early in April and they are to be found until late July. In some circumstances, here and there, *Aedes caspius*, *Aedes dorsalis* and *Aedes vexans* continue to be found as larvae and adults all the summer and their incidence usually increases in autumn. Otherwise, in some places in the south of the country, we found in autumn the larvae of *Aedes cantans* or *Aedes leucomelas*, species which are univoltine over the greatest part of their distribution area.

The overwintering anopheline females in the *maculipennis* complex leave their shelters and lay eggs in April, but sometimes they can do that even in early March if the temperature is high. The adults usually appear in mid May since the first larval generation develops slowly in spring or the first larvae in March do not survive subsequent frosts. The next generations succeed until late October when they enter animal shelters and other resting sites in households for overwintering.

In mid April, the first larvae of *Culex pipiens* appear in the outdoor breeding sites from eggs laid by the females after overwintering. They develop slowly so that the adults appear in mid May or even the end of May. In the urban environment, the form *molestus* develops continuously in the indoor breeding sites but generally at a rather low rate in winter. When the warm season comes, the form *molestus* breeds at a higher rate indoor but also it goes out and breeds in outdoor habitats together with the exophilic form of *pipiens*. So, beginning with July till late October, the populations of *Culex pipiens* containing both autogenous and anautogenous forms dominate the mosquito fauna in the urban environment. Populations, especially of the anautogenous form, this time together with anophelines of the *maculipennis* complex, dominate the mosquito fauna in rural environments.

Culiseta annulata can be found throughout the year as larvae and adults. In wintertime the larvae develop very slowly and the adults emerge in late March. On the Black Sea shore, from some mesothermal breeding sites with water temperature of 18°C, the adults emerge even in January at an air temperature of about 1°C.

Anopheles plumbeus, *Aedes geniculatus*, *Aedes pulcritarsis* and *Orthopodomyia pulcripalpis* develop in the wintertime in tree holes and the adults begin to appear in April. The larvae of *Coquillettidia richiardii* develop also in winter and in springtime and the adults of a single generation appear at the end of May and the beginning of June. There are daily variations of the flight activity of different species of mosquitoes depending on the weather, the habitat and other circumstances.

Most species carry on their flight activity at night to search for a host. Usually, they have two peaks of activity, the higher one during the nightfall and the smaller one at dawn. Among these species are *Anopheles maculipennis* s.l., *Culex pipiens, Anopheles hyrcanus, Aedes annulipes* and *Aedes flavescens*. The two peaks can be separated by a period of total lack of flight activity in the middle of the night as in *Coquillettidia richiardii, Aedes vexans* and other aedine species. Some species such as *Coquillettidia richiardii, Aedes vexans* and *Culex modestus* also have an intensive flight activity in the day time to find a host, this being usually more intensive than that in the night, at least in *Culex modestus* and *Aedes vexans* (Velehorschi *et al.*, 1990).

It is obvious that the mosquito fauna is specific for every type of ecosystem or habitat and also in every site it ceaselessly varies in time. On the other hand, it is very likely that the circulation of West Nile virus takes place in the south of Romania in both natural and rural or urban cycles established in different ecosystems and habitats, where different mosquito species could be involved in the virus amplification and transmission.

The introduction of West Nile virus in this territory seems to be a continuous process since the Delta and the meadow of Danube represent a particularly important station on the main migratory way of birds between Africa or other southern territories and the north of Europe. The virus settles afterwards in natural local cycles from which it is introduced in rural or urban cycles, this circulation involving different mosquito and bird species.

Culex pipiens seems to be the main vector for West Nile virus in the south of Romania, including Bucharest. It transmits the virus to humans from a rural or an urban cycle involving mosquitoes and birds. These cycles seem to go on in microfoci or microhabitats including the dwellings and households where mosquitoes carry on their life cycle and also birds (poultry and/or sparrows, turtledoves etc.) are present. So, the contact of humans with this vector seems to take place inside the dwellings or close to them both in the rural and urban environment. *Culex pipiens* is known as one of the vectors of West Nile virus in Israel and South Africa (Hayes, 1989).

Beside *Culex pipiens, Anopheles maculipennis* s.l. is also present in rural environments. Females concentrate inside the cattle shelters in high densities reaching several thousand individuals per shelter in some areas and periods of time. These species, and particularly *Anopheles atroparvus*, could play a part in rural environment as vectors since the West Nile virus was isolated from these mosquitoes in Portugal (Felipe *et al.*, 1990).

In some habitats and ecosystems where *Culex pipiens* is present, usually in low and very low densities, other mosquito species could be involved in amplification and transmission of West Nile virus. These species would be in the river and Danube meadows and in the Danube Delta: *Culex modestus*, which was the vector of West Nile virus in France, (Hayes, 1989) or *Coquillettidia richiardii* from which the virus was isolated in Czechoslovakia (Hubálek *et al.*, 1989). In other ecosystems even *Aedes cantans* and *Culex theileri* could be vectors, although they are spread in restricted areas in low densities in our territory. However the virus was isolated in Czechoslovakia from the first species and the second one is the most important vector of West Nile virus in South Africa (Hubálek *et al.*, 1989; Hayes, 1989). *Aedes geniculatus, Anopheles plumbeus* and *Aedes punctor* could be also suspected as vectors in some circumstances since the virus was transmitted by them following experimental infections (Hayes, 1989); and at least the first two species are spread over large areas of southern Romania.

In the south of Romania, which is a distinct ecological territory, the mosquito fauna includes at least one efficient mosquito vector of West Nile virus widespread in high population densities. This represents the most important factor determining the circulation of West Nile virus and the risk of virus transmission to humans. The serological investigations of domestic animals and of humans (Ungureanu *et al.*, 1988) confirmed the circulation of this virus in Romania.

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Anopheles cinereus Theobald 1901 and its synonym *hispaniola* Theobald 1903

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The first descriptions of *Anopheles cinereus* referred to material collected in Zimbabwe (Theobald, 1901) and Yemen (Patton, 1905). Those of *An. hispaniola* were of material from Spain (Theobald, 1903) and Algeria (Theobald, 1907). Differentiation is difficult, if not impossible (Mattingly & Knight, 1956), but the separate distributions of *cinereus* (Arabian Peninsula, Ethiopia and Sudan to Cape Province) and *hispaniola* (Mediteranean region, Equatorial Africa) (Knight & Stone, 1977) were assumed to meet in the central Africa region and Sahara, where local morphological variation was thought to represent the presence of intergrades (Gillies & de Meillon, 1968).

Senevet & Rioux (1960) concluded that the limited morphological differences warranted reducing the status of *hispaniola* to a subspecies of *cinereus*. Gillies & de Meillon (1968), whilst not disputing this reasoning, were more cautious and preferred to await further evidence.

Dahl & White (1978) consigned the name *hispaniola* to synonymy with *cinereus* and this action was acknowledged in the Addendum to the Second supplement of the *Catalog of the mosquitoes of the world* (Ward, 1984).

The only record of the taxon in Portugal was by Ribeiro *et al.* (1980), who compared their Portuguese specimens with the descriptions of *hispaniola* and *cinereus*. They concluded that morphological differences were insufficient for the two taxa to be regarded as separate species. Accordingly, they changed the ranking of *hispaniola* from species (not synonym) to subspecies of *cinereus*, making no mention of the prior synonymy by Dahl & White, which they appear not to have seen. This action was acknowledged in theThird supplement of the *Catalog of mosquitoes of the world* (Ward, 1992).

Morphological variation occurs in some of the characters mentioned in the original descriptions of *cinereus* and *hispaniola* and also in other characters (Raffaele & Coluzzi, 1961; Gillies & de Meillon, 1968; Holstein *et al.*, 1970; Ribiero *et al.*, 1980; Ramsdale & de Zulueta, 1983; Ramsdale, 1991). This variation is not confined to populations from opposite ends of the combined distribution. Population genetic studies (cytogenetic and/or iso-enzyme analysis) may eventually show that *cinereus* is a species complex. No such work has yet been undertaken but there seems to be general acceptance that these nominal taxa cannot be reliably separated on morphological grounds.