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MOSQUITOES

Epidemiology of West Nile infection in Volgograd, Russia, in relation to climate change and mosquito (Diptera: Culicidae) bionomics

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Abstract In 1999, there was the large outbreak of West Nile fever (WNF) in Southern Russia (>500 cases in the Volgograd Province). In 2000–2004, the WNF incidence rate decreased steadily to zero, but a new outbreak occurred in 2007 (64 cases). The analysis of historical climate data for Volgograd from 1900 to present showed that the years 1999 and 2007 were the hottest ones due to a very mild "winter" (Dec.–Mar.) and a hot "summer" (June–Sep.). There are up to 15 potential WNF vectors in Volgograd, but only *Culex pipiens* and *Culex modestus* are abundant in late summer, both in urban and rural settings. Only these species are naturally attracted to and feed on both humans and birds. The RNA of pathogenic WN virus genovariant was found by reverse transcriptase polymerase chain reaction only in *Culex* mosquitoes at the infection rate of about 0.04%. So these species may be considered as potential WNF "bridge vectors" between birds and humans as well as main vectors in sylvatic avain cycle. Their abundance in an epidemic season was higher in the years with a mild winter and a hot summer, so this phenomenon may serve as a connecting link between a climate and WNF epidemiology. These findings give some hints on the predisposing factors for WNF epidemic as well as the possibility to predict WNF outbreaks in the temperate climate zones.

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Introduction

West Nile fever (WNF) is a severe mosquito-borne flaviviral infection, first discovered in tropical and subtropical regions. It was assumed that, in Russia, the potential area of WNF is limited to the southern regions, where the annual sum of "daily effective temperatures" (higher than 10°C), or in another term, "cumulative degree days at threshold of 10°C," is greater than 2,800°C (Fig. 1). Indeed there were findings of WN-specific antibodies in healthy adult donors and in animals, for example horses, in these regions. Nevertheless, until late 1990s, human clinical cases were diagnosed only in the Astrakhan Province, where the annual sum of "daily effective temperatures" might reach 3,800°C. Theoretically, there are several restricting factors for WNF in the temperate climate zone, namely the successful WN virus transmission depends on the abundance of competent vectors, first of all, *Culex* mosquitoes, and their ability to survive in winter and to propagate in a warm period. The rate of WN virus multiplication in mosquitoes may be also of importance. Practically, after the introduction to Western hemisphere, WN infection colonized shortly all territories of the USA but did not settle in the northern part of the American continent (Gubler 2007). The restriction factors are temperature-dependent and so climate-dependent. Therefore, the WN infection is a proper model for the understanding of possible global warming effects. However, the climate records were not long-term, and the proper diagnostics and registration of WN disease was absent until the last decade. Possibly, it is more wise to consider the direct impact of daily and monthly weather on WNF epidemiology rather than the effects of climate trends. In this paper, we shall try to do that by the example of Russian Volgograd Province (Fig. 1), where the largest WNF outbreak in Eastern

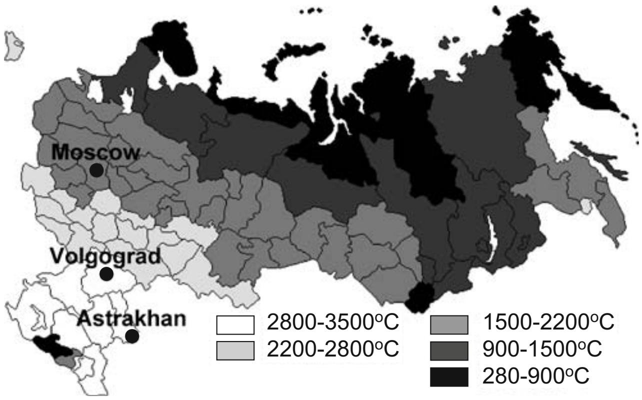


Fig. 1 Annual cumulative degree days at threshold of 10°C (DD10) in 1990–1995 in Russia. The area with DD10>2,800°C is assumed to be endemic for WNF. WN virus may be occasionally introduced by migrating birds into the area with DD10>2,200°C if a summer is sufficiently hot. There is no condition for WN virus spread in the area with DD10<2,200°C

hemisphere was observed in 1999 (Platonov 2001; Platonov et al. 2001).

Materials and methods

We used three climatic datasets for Volgograd City. The first type of datasets was obtained from the web site of International Research Institute for Climate Prediction http://iri.columbia.edu and contained the monthly data on mean temperature (M-TEMP) from 1901 to 1990 and on precipitation (M-PREC) from 1918 to 1988 in Volgograd. The second dataset contained the daily data on mean temperature (D-TEMP), precipitation (D-PREC), and mean humidity (D-HUMI) from 1996 to 2001. This dataset was obtained from the Volgograd Center for Sanitary and Epidemic Control. The average values of M-TEMP and M-PREC for these years were calculated using this dataset. The third dataset was obtained from the web site http://meteo.infospace.ru and contained the data on temperature (T-TEMP) and humidity (T-HUMI), recorded eight times per day from 1999 to 2007. The values of D-TEMP, M-TEMP, D-HUMI, and M-HUMI for these years were calculated using this dataset; they coincided with former dataset, where both datasets overlapped. All the datasets were sourced from the reports of local meteorological services (Platonov 2006).

The number of human clinical cases of WN disease in the Volgograd Province is based on the reports of the Volgograd Center for Sanitary and Epidemic Control. Since 2000, the serum samples from all patients with meningitis or encephalitis and presumably from all hospitalized patients with fever were studied by WN-specific IgM-capture enzyme-linked immunosorbent assay (ELISA; Savtchenko et al. 2007). The studies in 1999 were presented in detail elsewhere (Platonov 2001; Platonov et al. 2001); about 70% of possible WNF cases were laboratory investigated in 1999. The estimations for 1997 and 1998 were based on the serological retrospective investigation of patients who contracted aseptic meningitis in these years (Platonov 2001). The population of the Volgograd Province was about 2,700,000, but 93% of WNF cases were related to the capital Volgograd City and its suburb Volzskii with about 1,300,000 inhabitants in total (Savtchenko et al. 2007).

The data on mosquito's bionomics are based on our own field studies in Volgograd Province in 2002–2007. The entomological methods were described in detail elsewhere (Fyodorova et al. 2006; Fedorova et al. 2007; Lopatina et al. 2007; Platonova et al. 2007). Briefly, outdoor mosquitoes were sampled by several methods. The drop-net was used for sampling host-seeking females attracted to an investigator. Lard-can traps, with chicken as bait, were used in collecting ornitophilic mosquitoes. Light trap and gravid mosquito trap were used at several sites to investigate the species diversity. Mosquitoes at the resting sites outdoors and mosquitoes indoors were collected with a backpack aspirator. All indoor sites in Volgograd were multistory buildings; the main entryways, staircases, and basements were sampled. At outdoor sites, mosquitoes were aspirated from hollows, ground cavities, and among vegetation surrounding marshes and streams. Aspiration collections also were made in henhouses and cattle sheds. The samples were taken from May to August. For the analysis of mosquito bloodmeal hosts, we have collected 690 female mosquitoes belonging to 11 species in 2003 and 1,314 female mosquitoes belonging to 12 species in 2004 in Volgograd region. The individual midguts of blood-fed mosquitoes were squashed on to filter paper. Bloodmeal was identified by means of precipitation test with antisera against human, chicken, cattle, dog, pig, horse, and rabbit blood.

Results

WNF human cases were not revealed in the Volgograd Province before 1999. In 1999, there was the large outbreak of mosquito-borne WNF in Volgograd City and its vicinity. More than 500 human cases were registered in the population of about 1,300,000, that corresponded to about 25 cases per 100,000 inhabitants. Ninety-five WNF cases were found in the Astrakhan Province in 1999. Also, WNF human cases were first diagnosed in Krasnodar Province (year 1999) and Rostov Province (year 2000), located not far away. WNF was still registered in southern Russia after 1999, but the WNF incidence rate declined steadily from two cases per 100,000 to zero in 2001–2004. Smaller new WNF outbreak (64 WNF cases) occurred in 2007 (Table 1).

Table 1 The number of human clinical cases of West Nile disease in the Volgograd Province in relation to weather

Year	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Number of patients with WND confirmed by IgM-capture ELISA	>5	>35	>380	32	15	14	0	0	4	11	64
Mean temperature in “winter”, December-March			−1.7	−0.9	−2.2	−1.8	−8.0	−1.5	−3.9	−6.1	−0.4
Number of hours, when the temperature was below −9°C in the preceding winter			183	324	192	369	1,125	150	348	642	222
Mean temperature in third quarter, July–September			22.3	19.8	21.3	22.0	19.5	20.7	21.2	21.8	22.9
Number of hours, when the temperature was above 25°C			918	585	693	750	453	567	741	897	1,065
Mean humidity (%) in second and third quarters			51.3	61.5	56.0	49.0	59.0	58.5	56.3	54.5	46.9

WNF epidemic season in Volgograd lasted from late July to early October with a peak at the end of August.

Weather in Volgograd

The city of Volgograd (48°48′ N, 44°42′ E) stretches along the west bank of the Volga river. On the west and northwest, it is surrounded by a chain of hills and steppe. The extended wetland is located on the east bank of Volga river opposite to the city (Fyodorova et al. 2006). The climate is continental; there are cold winters (M-TEMP below zero, up to −22°C) and hot summers (M-TEMP is usually 20–25°C; Fig. 2). Most months in 1999 and in 2007 were rather warm in comparison to the usual values (the circles and squares lie above the boxes showing the interquartile ranges of the distributions).

When we calculate mean annual temperature (as the mean of mean monthly temperatures), the finding is even more striking. Year 1999 was the hottest year in the twentieth century (Fig. 3).

The temperature effects on virus propagation, host and vector ecology may differ between seasons (Fig. 2). So we analyze the same data, stratifying by quarters or seasons. The quarter box-and-whisker plot (Fig. 4) demonstrates that mean quarter temperature was very high, nearly maximal, in first and third quarters of 1999 and 2007 but moderately high, nearly at upper quartile level, in second and fourth quarters.

The possible interpretation of this phenomenon is that the warm temperature in first quarter supports the survival of overwintering vectors, and the high temperature in third quarter facilitates the growth of virus in mosquitoes, as well as

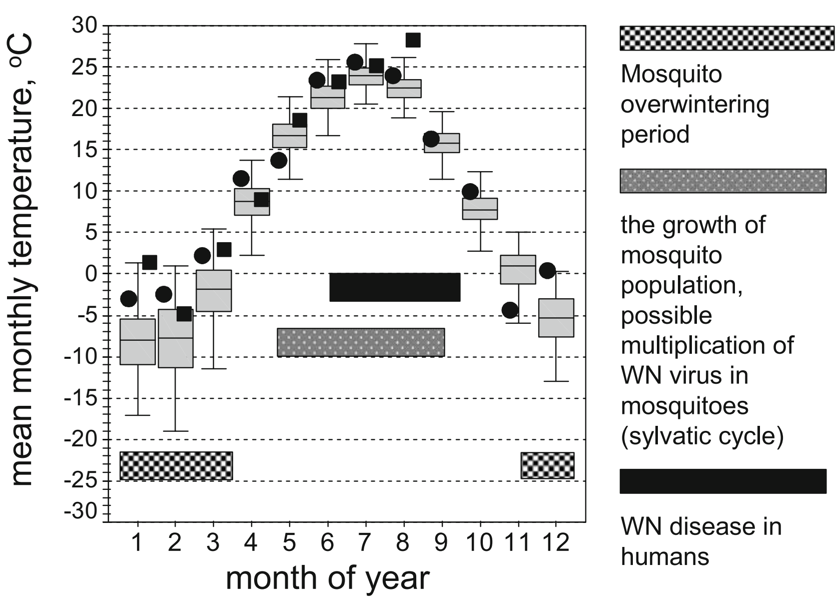


Fig. 2 Box-and-whisker plot of monthly temperatures (years 1901–2007) and the monthly temperature in 1999 (*circles*) and in 2007 (*squares*) in Volgograd

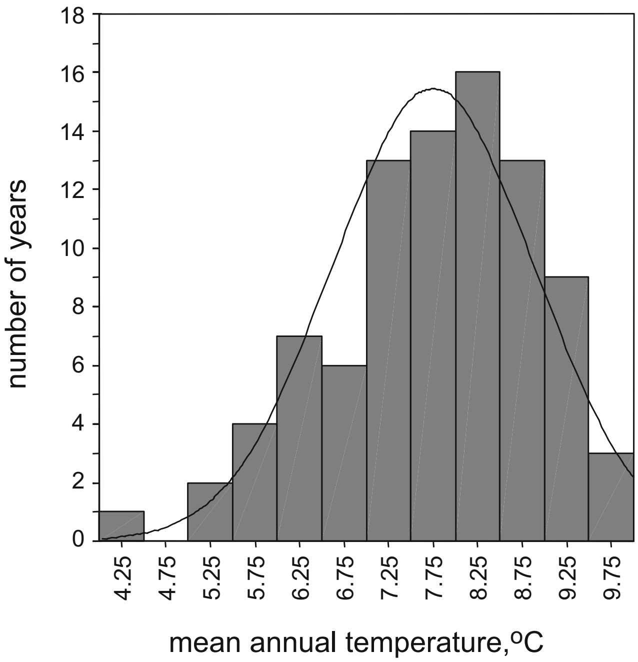


Fig. 3 Histogram distribution of mean annual temperatures in 1901–2007 in Volgograd. The annual temperature was higher than 9.5°C only in years 1999, 2002, and 2007

propagation of mosquitoes themselves. Perhaps, it is more exact to mark off a "winter season" (December–March) and a "summer season" (June–September) in Volgograd settings. The mean season temperatures in individual years are shown on the temperature scatter plot in Fig. 5. The conventional threshold temperatures for "beneficial for WNF condition" in Russia was assumed to be −2°C in "winter" and 22.5°C in "summer". The mean temperatures were above one of these thresholds in several years, but both conditions coincided only in years 1999 and 2007.

The standard WNF surveillance and detailed weather datasets for 1999–2007 provides the possibility for additional analysis (Table 1). The annual number of WNF cases correlates significantly with the mean temperature in "winter," December–March (Spearman correlation coefficient R=0.59), with the mean temperature in third quarter, July–September (R=0.67), with the number of hours when the temperature was above 25°C (R=0.70), with the mean atmospheric pressure in third quarter (R=−0.71), and with the mean humidity in second and third quarters (R=−0.51). At that, the mean humidity correlates negatively but highly with the temperature in third quarter (R=−0.97).

So the years with warm winter and hot summer, as 1999 and 2007, are most suitable for WNF outbreaks in Volgograd (Table 1, Fig. 6). Very cold and long winter, as in 2003 and 2006, is incompatible with the large number of summer WNF human cases. It seems that there is also a kind of aftereffect. The weather in 2000 and in 2004 were similar, but the former weather succeeded to the outbreak in 1999, and the latter weather succeeded to the suppression of WN virus transmission in 2003. That might explain the higher number of cases in 2000 than in 2004–2005 (Table 1, Fig. 6).

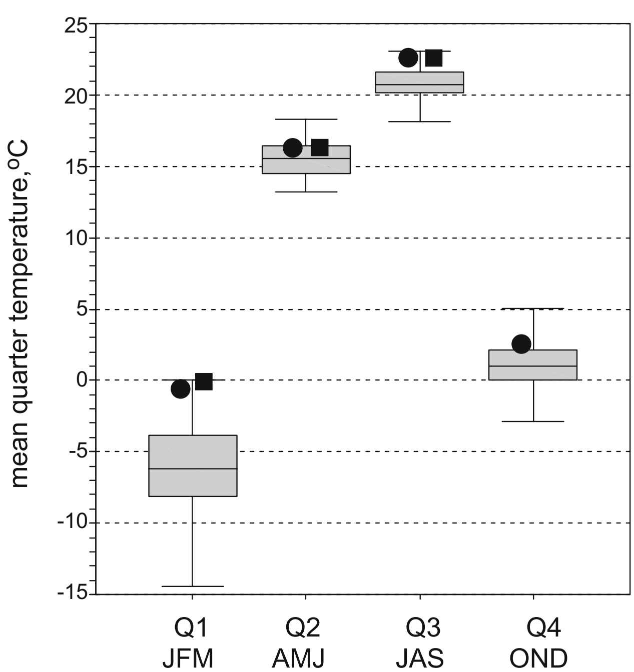


Fig. 4 Box-and-whisker plot of quarter temperature (years 1901–2007) and the quarter temperature in 1999 (*circles*) and in 2007 (*squares*) in Volgograd. *Q1, JFM* Quarter 1, January–February–March, etc

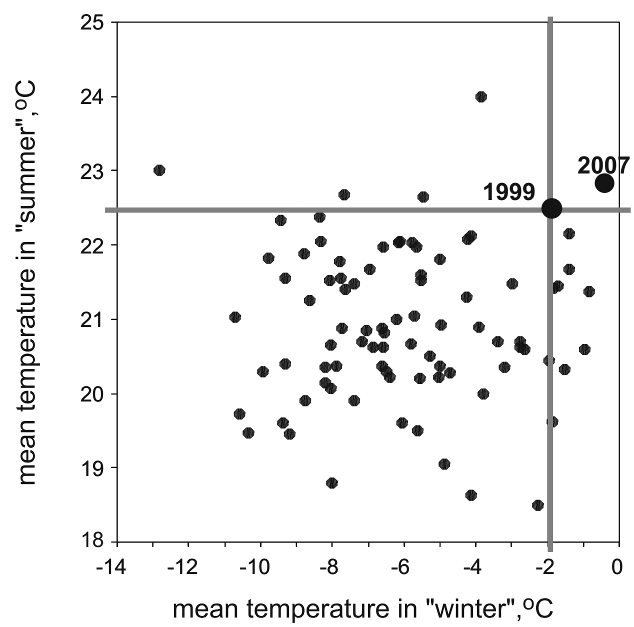


Fig. 5 Scatter plot of mean season temperatures in Volgograd in 1901–2007. Each *point* corresponds to a year. The conventional threshold temperatures for "beneficial for WNF condition" are shown by *grey lines*. A "winter season" is December–March, and a "summer season" is July–September in Volgograd

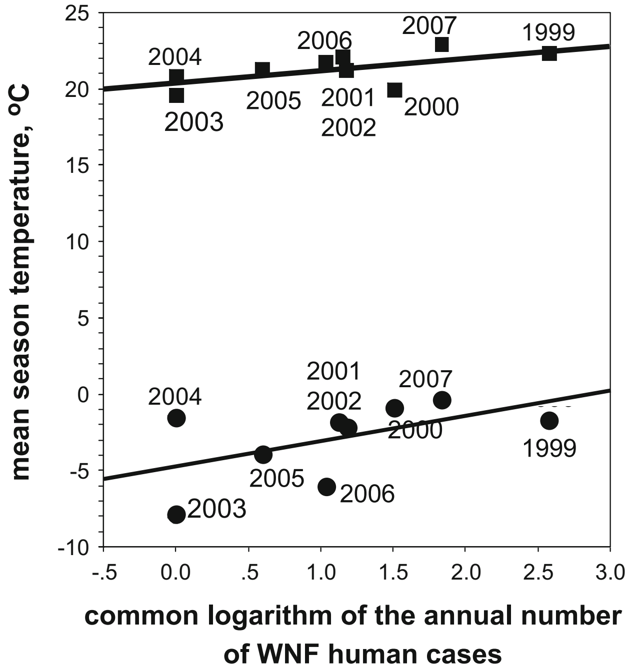


Fig. 6 Scatter plot of mean temperatures and West Nile fever incidence in Volgograd in 1999–2007. Each *point* corresponds to a year. *Abscissa* Common logarithm of the annual number of West Nile fever human cases; *ordinate* the mean temperature in December–March ("winter season," *circles*) and July–September ("summer season," *squares*)

The epidemiology of WNF is a very complex phenomenon, including human behavior and mosquito control measures, avian bionomics, WN virus virulence, and pathogenicity, but probably, the WN vectors' bionomics is mostly climate- and weather-dependent.

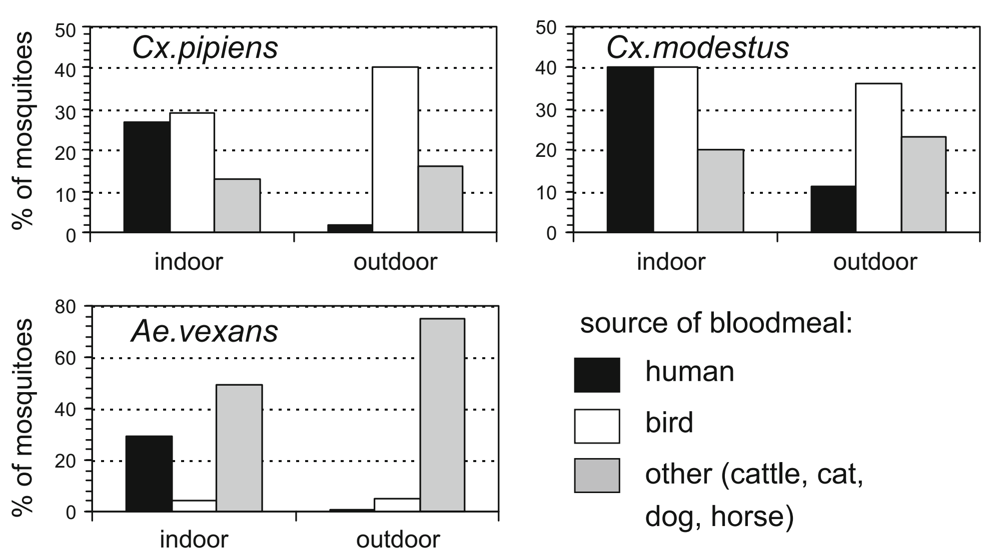
Assessment of potential West Nile virus vectors in Volgograd

There are up to 15 mosquito species in Southern Russia, which may be potentially the WN virus vectors (Fyodorova et al. 2006; Gubler 2007; Fedorova et al. 2007; Lopatina et al. 2007; Platonova et al. 2007; Hamer et al. 2008). In this paper, we shall focus on several most abundant species. In June–July indoor collection, the floodwater mosquito *Aedes vexans* (Meigen) and *Culex pipiens* Linnaeus accounted for about 90% of specimens collected. In August indoor collections, *C. pipiens* was the dominant species (≈85%). June–July outdoor collections were more homogeneous in Volgograd City, with each of five species representing 10–35% of the specimen collected: *Culex modestus* Ficalbi, *Aedes caspius* (Pallas), *A. vexans*, *Aedes cinereus* (Meigen), and *Aedes flavescens* (Mueller), whereas in rural sites, *A. vexans* dominated (≈75%). In August outdoor collections, the summer generations of multivoltine species of *C. pipiens* were most frequent in Volgograd City (≈60%) and presented in approximately the same proportion (≈25%) as *C. modestus* (≈30%) in its rural vicinity. Collections made in livestock shelters were dominated by *Anopheles messeae* Falleroni both in June–July and August (≈90%).

One of the tasks of entomological investigation was to reveal the feeding preferences of different mosquito species in different biotopes (Fyodorova et al. 2006; Platonova et al. 2007). The results for most abundant species of *Culex* and *Aedes* genera are presented briefly (Fig. 7).

In Volgograd conditions, *Culex* mosquitoes, first of all *C. pipiens*, are mainly ornitophilic, as it might be expected. However, the substantial part of indoor population collected in July–August fed on humans. So this species may be

Fig. 7 Bloodmeal host utilization of mosquitoes collected indoor and outdoor in the Volgograd Province



considered as potential WNF "bridge vector" between birds and humans as well as main WNF vectors in sylvatic avain cycle.

Outdoor *Aedes* mosquitoes fed mainly on cattle. A part of *Aedes*, including *A. vexans* collected indoor, fed either on humans or on cats/dogs. The findings of avian blood in *A. vexans* were rare, but one could not exclude the role of this species in WNF virus transmissions because of its abundance in June–July.

Mosquitoes belonging to *Anopheles* genus and its prevalent *A. messeae* species fed mainly on cattle, even those mosquitoes collected in a henhouse. The small proportion of *Anopheles* bites either humans or birds in urban conditions or in a henhouse, but the potential transmission role of this subpopulation is unclear. Epidemiological data obtained in 1999 and later did not reveal the work at a henhouse or the rural habitation in general as a risk factor for clinical human WNF cases.

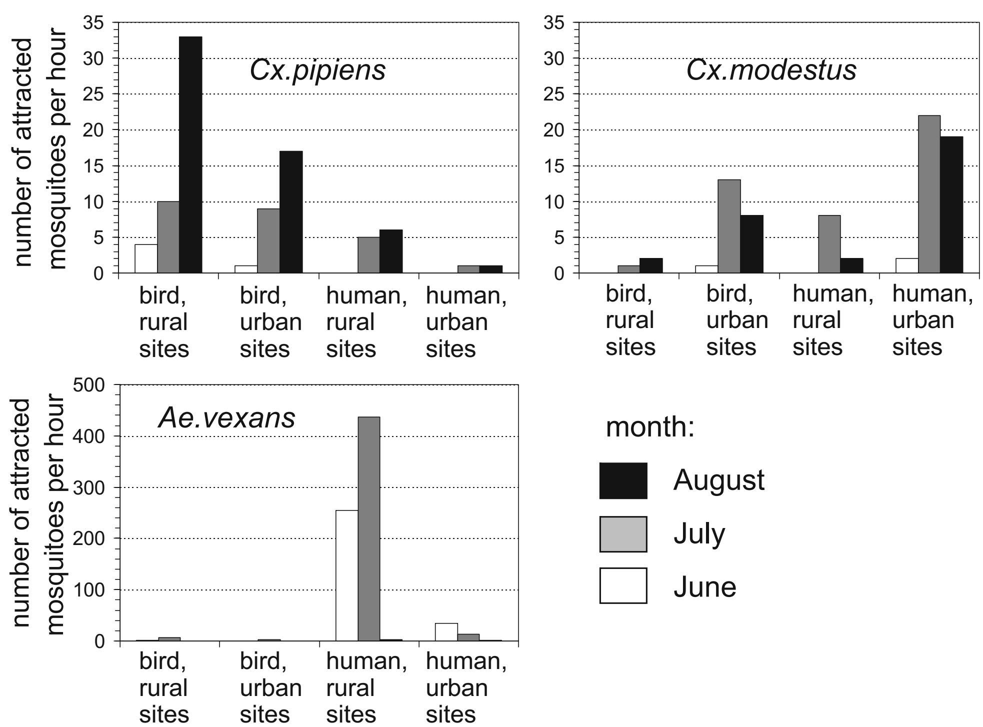
These data were supplemented by trapping of host-seeking mosquito females (Fig. 8). *Aedes* mosquitoes attacked humans very intensively in June–July in rural sites. The number of *Culex* mosquitoes increased from June to August; ornitophilic *C. pipiens* was prevalent in rural sites, and *C. modestus* was prevalent in Volgograd City outdoors. The latter species was more attracted to humans.

Twenty-two thousand five hundred female mosquitoes in 606 pools were tested for the presence of WN virus RNA by reverse transcriptase polymerase chain reaction (Fyodorova et al. 2006; Platonov et al. 2001). Mosquitoes belonged to 13 species and were collected in 2001–2003. Maximal likelihood infection rate estimates per 1,000 females of *C. pipiens* was equal on 0.51 with 95% confidence interval (CI) from 0.13 to 1.4. The infection rate estimates per 1,000 females of *C. modestus* was 0.24 (95% CI=0.014–1.2). Sequencing of amplicons confirmed that all WN virus isolates from *Culex* mosquitoes belong to the same pathogenic WN genotype which was permanently found in patients and crows in 1999–2003 in Volgograd Province. Pathogenic WN virus was not detected in other species including 2,763 *A. messeae* specimens and 7,173 *A. vexans* specimens.

Taken together, these data indicate that the weather influence on WNF epidemiology in Volgograd may be realized via the influence on *Culex* mosquitoes.

In Volgograd conditions during a cold winter, the mortality among overwintering mosquitoes exceeds 90%. "Very cold period" (less than −9°C) varied from 200 h in 1999 and 2007 to 1,100 h in 2003 (Table 1). The development of autogenous *C. pipiens* overwintering in non-heated basements is also affected by an ambient temperature. The summer ambient temperature influences the lengths of the gonotrophic cycle, which is about 6 days at 18–20°C but decreases to 4 days at 22–25°C and 3 days at 27–29°C for *C. pipiens* (Ii'chenko 1974). As a result, the number of *Culex* mosquitoes grows faster in a hot summer than in a cooler one (Fig. 9). In addition, a shorter gonotrophic cycle implies increased daily biting frequency and thus increased vectorial capacity. Last but not least, the extrinsic incubation period of the WN virus in *Culex* mosquitoes, as well as their ability for WNV transmission,

Fig. 8 The number of host-seeking mosquito females trapped per hour by using the drop-nets and lard-can traps



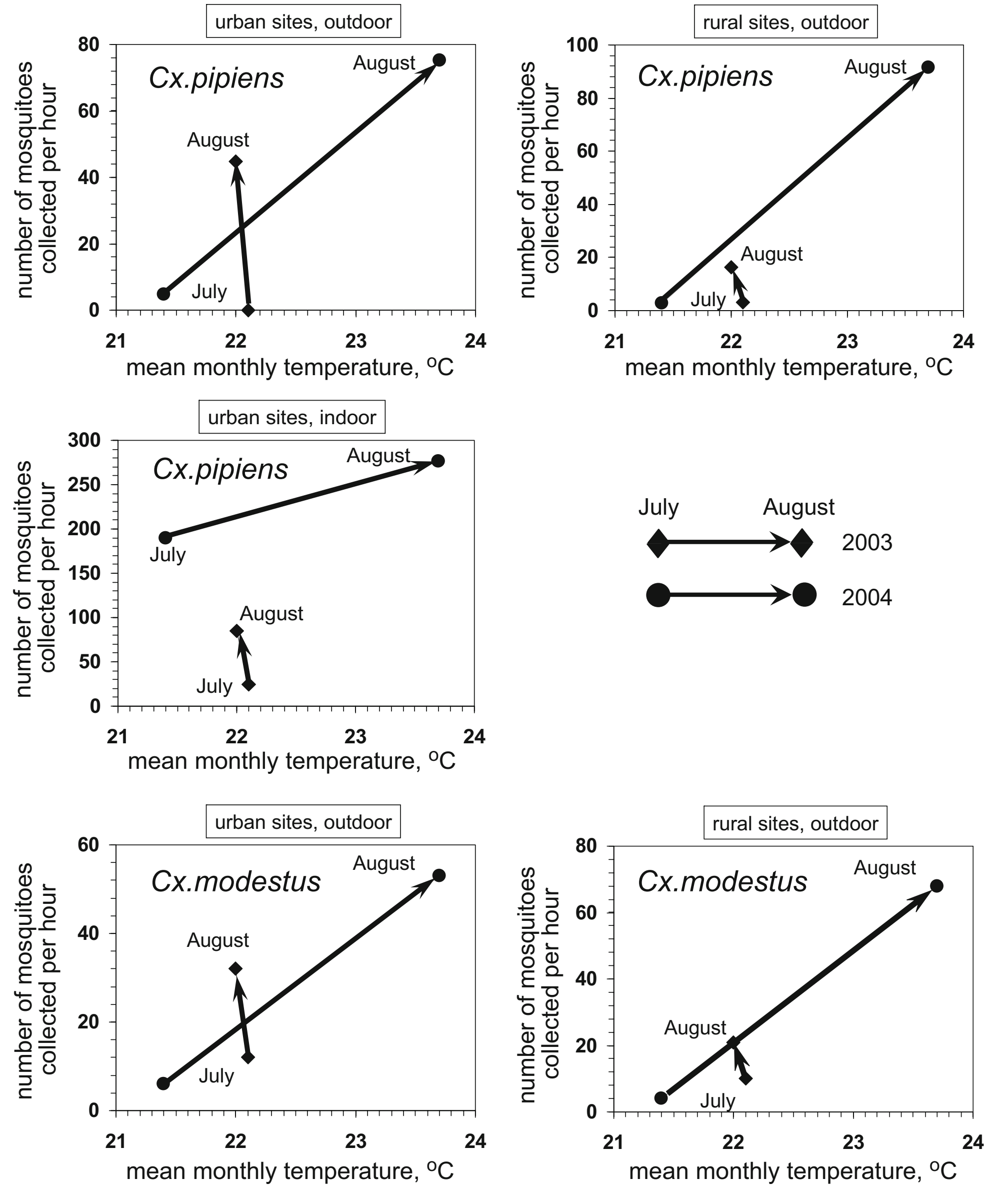


Fig. 9 The number of *C. pipiens* and *C. modestus* mosquitoes collected by backpack aspirators in different habitats. The *arrows* indicate how the mean monthly temperature and the number of mosquitoes change from July to August

is highly temperature-dependent (Dohm et al. 2002; Reisen et al. 2006). Reisen et al. (2006) concluded that "The invading NY99 WNV strain therefore required warm temperatures for efficient transmission.…Temperatures in the United States during the epidemic summers of 2002–2004 indicated that WNV dispersal and resulting epicenters were linked closely to above-average summer temperatures." Number of hours when the temperature was above 25°C reached 900–1,100 h in epidemic years 1999 and 2007 in Volgograd and was significantly lower in other years (Table 1).

Some features of mosquito behavior may also vary. Our observations showed that in Volgograd, the anautogenous *C. pipiens* originated outdoors would readily use multistory buildings as resting sites in summer; both unfed and blood-engorged females were collected indoors. To the contrary, the populations of autogenous *C. pipiens* developed in underground habitats, namely in flooded basements, but might go into the open habitats. This counterflow is minor in a cold summer, while in a hot summer, the phenomenon becomes evident. Thus, the ornitophilic mosquitoes bite humans, and the mainly anthropophagic form starts attacking birds, and both forms enhance their ability to serve as a bridge vector for WN virus.

Discussion

Common opinion is that the real or potential global warming may basically influence the emergence of vector-borne diseases. Some authors suggest that "climate change is probably the most important requirement for the emergence of arthropod-borne diseases such as dengue fever, yellow fever, Rift Valley fever, Japanese encephalitis, Crimean-Congo hemorrhagic fever" in Europe (Gould et al. 2006). Other specialists argue that "demographic and sociologic factors also play a critical role in determining disease incidence" (Gubler et al. 2001). We are ready to agree with Kovats et al. (2001): "the literature to date indicates that there is a lack of strong evidence of the impact of climate change on vector-borne diseases….New approaches to monitoring…are necessary in order to provide convincing direct evidence of climate change effects." But the WNF may be an exception and provides some evidence, at least, of weather effects. Paz (2006) stated that, in 2000 in Israel, "The minimum temperature was found as the most important climatic factor that encourages the disease (WNF) earlier appearance. Extreme heat is more significant than high air humidity for increasing WNF cases. An early extreme rise in the summer temperature could be a good indicator of increased vector populations." Savage et al. (1999) reported that the transmission season of the epidemic WNF year 1996 in Romania was significantly drier but not hotter than the preceding years (16). El Adlouni et al. (2007) stated that the major human WNF epidemics reported in the northeast region of North America in 2002 was related to unusual climatic conditions which could be observed once every 40 years on average. Hot epidemic summer was characterized by the cumulative degree days at threshold at 25°C, and mild preceding winter was characterized by the cumulative degree days above −5°C in November–March; these parameters resemble ours used in Table 1.

We believe that the weather effects are most critical in temperate climate at the border of WNF area, for example in Volgograd (Revich et al. 2003). Successive warm years may enhance, little by little, the regional WNV circulation; on the contrary, very cold winter may interrupt the local transmission and require the new introduction of WNV by migrating birds. Indeed, in 1999–2003, we found the same "Volgograd" genovariant of pathogenic WN virus in patients, birds, and *Culex* mosquitoes (Fyodorova et al. 2006; Platonov 2001; Platonov et al. 2001, 2002). This genovariants belonged to so-called lineage I. In 2007, we detected in human brain and blood samples the RNA of WNV belonging to lineage II; to date, there is no other report on clinical cases caused by lineage II WNV in Europe (Bakonyi et al. 2006).

The preparedness for mosquito-borne disease outbreak and the proper mosquito control are also important. WNF had never been registered before 1999 in the Volgograd Province, which is why local specialists did not operatively recognize the 1999 outbreak and no anti-mosquito measures were taken before September 1999 when several hundreds of people were already infected. Many basements in city buildings were infested with *Culex* mosquitoes, which is a known risk factor for acquiring WN infection (Han et al. 1999). Since 2000, more active measures have been taken in order to eliminate domestic and peridomestic mosquito breeding sites and reduce domestic and peridomestic mosquito exposure in Volgograd. For example, in 2007, more than 6,000,000 m^2 or about 5,000 basements were subjected to anti-mosquito treatment. So, despite the fact that year 2007 was even warmer than year 1999, the WNF incidence rate was ten times less in 2007.

In conclusion, our findings give some hints on the predisposing factors for WNF epidemic as well as the possibility of weather surveillance and prediction of WNF outbreaks in the temperate climate zones such as southern Russia. Entomological studies stress the need of vector control, first of all, the control of *Culex* mosquitoes both in their breeding and resting sites indoors and outdoors.

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