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INVESTIGATIONS ON WEST NILE VIRUS (WNV) CIRCULATION IN THE EPIDEMIC SEASON 2007

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ABSTRACT. Our studies were designed to explore the impact that environmental changes and other causes have at the re-emergence of human infections with West Nile virus (WNV). Have been identified, evaluated and categorized the risk ecosystems in Romania and environmental conditions caused by global changes that may influence the spatial-temporal distribution and dynamics of WNV. Were conducted complex investigations of WNV transmission cycle in different types of ecosystems in Romania, integrating data of evolution of the environment, with particular emphasis on climate change and other environmental changes. We followed the level present of WNV in its vertebrate hosts and vectors in different ecological conditions. Was completed GIS database of land cover maps (LC) in the investigated areas in order to achieve good spatial analysis and to integrate data on WNV circulation, with their exact location.

Keywords: hosts, transmission cycle, climate change, land cover/ land use, GIS database

INTRODUCTION

To characterize the weather conditions that could favour WNV circulation in 2007 were considered essential climatic factors (temperature and precipitation) from previous years in the study areas: daily air temperature and daily amount of precipitation, monthly averages air temperature, temperature average maxima, temperature average minima, maximum temperature and absolute monthly precipitation amounts. Climatic data from previous years of 2007 were compared with their average values over the period 1961 to 2006, like the reference period.

Our team has made the detection of WNV in the life cycle, both vectors (mosquitoes) and the main hosts (wild and domestic birds) and secondary or tangential (mammals -horses and humans).

To highlight the types of ecosystems in the study areas we comparing the Land Cover / Land Use - LC / LU maps in 2000 and 2003.

MATERIALS AND METHODS

WNV is transmitted by vectors to tangential hosts (after the blood was taken from the main host), to mammals, including man but at this stage it cannot be resumed vectors.

Investigation and surveillance of WNV circulation included simultaneous tracking of viral activity levels in the vertebrate hosts and vectors.

Were investigated the virus present in vector populations and in the vertebrate hosts (including at humans) in the context of climatic and other factors in 2007 and in the study areas to detect and forecast changes in the dynamics of the arbovirus transmission. Investigations led to a precise understanding of the biology, ecology, the interactions between vertebrate hosts, vectors and biological and environmental factors that influence arbovirale cycles.

Our investigations on WNV circulation in the natural and anthropogenic ecosystems in the study areas included three (3) main directions:

- Serological study of main vertebrate hosts of WNV transmission cycles – domestic birds from rural and urban outbreaks and wild birds in natural and anthropogenic ecosystems.
- Serological study of horses (tangential hosts) from rural and urban outbreaks of WNV. The horses are the best indicators of the circulation level of this virus because they live near humans where there is the highest risk of transmission of WNV.
- Study on vectors (mosquitoes) from natural and anthropogenic ecosystems and detection and isolation of virus from these insects.

Study of seroprevalence of antibodies against WNV was performed by different ELISA techniques, suitable for the investigated hosts:

- ELISA test for capture of IgG in domestic birds (Ebel et al., 2002);
- Epitope-Blocking ELISA test for detection of antibodies of WNV in different species of wild birds (Blitvich et al., 2003);
- ELISA test for capture of IgG in horses.

The capture of mosquitoes was achieved by using traps (light CDC traps, Reiter traps to capture the pregnant mosquitoes females, traps with bait - bird) and entomological net to capturing on the vegetation

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and humans and also to capture from anthropogenic places (artificial shelters - animal shelters, housing).

Attempts virus detection were performed using RAMP immunological and RT-PCR test and virus isolation by intracerebral inoculation in infant mice.

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Land Cover Classification System - LCCS is a comprehensive methodology for description, characterization, classification and comparison of different types of land covers. Land Cover/Use maps (LC/LU) were performed using data obtained from satellite imagery interpretation, compared with topographic and/or thematic maps.

Satellite imagery used to obtain LC/LU maps were provided by these satellite systems:

Satellite system	Image acquisition	Spatial resolution
Landsat – 7 ETM	May – July 2000	15 m – panchromatic 30 m - multispectral
Landsat – 5 TM	June – August 2003	30 m - multispectral
Landsat – 5 TM	June – July 998	30 m - multispectral
Quick Bird	June 2003	0,6 m

Landsat satellite - 5 TM during June-August 2003 provided imagery for the following dates (06. 24, 07.01, 07.26, 08.02, 08. 07, 08.09, 08.14, 08. 25).

Topographic and thematic maps used were:

Maps	Scale	Type	Covering
Topographic maps	1:50 000	Raster – black/white	All country
Topographic maps	1: 100 000	Raster – Color	All country T
Thematic maps	1: 200 000	Raster – Color	Danube Delta
Thematic maps– geomorphologic, hydrological and vegetation	1: 500 000	Raster – Color	All country

The LCCS maps have been made with software ERDAS Imagine, ArcView, ArcGIS and LCCS using the following layers:

- satellite imagery - multispectral
- satellite imagery - panchromatic
- topographic maps 1:50.000 and 1:100.000 scale
- vegetation index - NDVI (Normalized Difference Vegetation Index) - a derivation of satellite imagery
- vectorised map of land cover according to FAO LCCS.

The land cover classes from the database are in the number of 68 and grouped into 10 categories (with English nomenclature to preserve the unity of LCCS).

The LCCS/ LU maps make done by implementing the FAO land cover classification system.

The vectors was performed at scale 1:50.000 adding additional data through field visits and using HR (HR - High Resolution) or VHR (VHR - Very High Resolution) images.

RESULTS AND DICUSSIONS

Investigations on WNV circulation in the epidemic season 2007

Characterization of climatic factors from 2007 in Romania

In the last century, in Romania, the climate was characterized by constant increase of the average temperature, average heating at 0.3 ° C. This process was stronger in the Eastern region of Romania (in Bucharest, average heating was 0.8 ° C and about as high in the rest areas, including Moldova).

The annual average temperature in 2006 was by 0.1 º C on whole country above the average for the period 1961- 1990. During April-December was warmer than the reference period, with 0.1 to 1.2 ° C (the mean increase in December was 1.8 ° C).

In 2007 especially in the Middle East of Romania were recorded temperatures above the average of the last century.

In the regions from South and East, during July-August the temperature was 35 - 42 º C. In this year was recorded (for the second time in the last 150 years)

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the temperature of 44.5 º C in Bucharest and in some settlements in S of Bucharest, near the Danube.

This year was an extremely dry year (in the last 100 years) in the East and South of country.

The causes were almost total absence of rainfall from September 2006 - the first half of August 2007 and consistently the high temperatures from April 2006 - August 2007 in these areas.

These climatic conditions influenced the mosquito's fauna in different areas and times.

Study areas of WNV circulation

Since the last human cases of WNV infection in Bucharest Metropolitan Area (BMA) were recorded in 2004 and 2005 and in Tulcea County in 2001 and that the mosquito fauna was extremely low in 2006 and very low in 2007, our team extended the investigation areas of virus circulation on vectors and hosts in another counties of Romanian Plain and on South of Moldova region (was identified a human case in 2007).

Human infections with WNV

Special climatic conditions of Romania in 2007 influenced the fauna of mosquitoes' vectors of WNV.

Mosquitoes' fauna was extremely low in all country especially in the middle part of the South. Even in the Danube Delta and in the lagoons area, the size of mosquito populations was much lower than in other years. Mosquitos' populations began to increase slowly in August when some rains began to fall. This extremely low abundance of vectors is explaining the low number of human cases of the West Nile virus (WNV) in this year.

The two human cases of WNV neurological infections occurred in localities: Vădeni (Prut Valley) – Galati County and Greceanca – Buzau County. The very low number of human cases has been linked to extremely low abundance of mosquito vectors to their total absence in different places and times of the year.

In terms of the number of infections with this virus, in the years after the epidemic of 1996, Galati county is the second area after Bucharest (with its suburbs), with a large number of human cases.

The 2007 is the second year after the outbreak of 1996 with the lowest number of WNV human cases in Romania.

In 2006 and 2007 there were 2(two) human cases in each year, because there were unusual climatic factors that induced extremely low abundance of mosquito populations. A small number of cases, only 3 (three) was recorded in 2002, a very dry year, when mosquito populations were also very low.

After the outbreak of 1996 continued to appear annual human cases of WNV infection, so it was a total of 92 by 2008, registering 6.5% mortality in this period (1997-2008).

The human cases from 1997 - 2007 have appeared on a very large territory, including in particular the Romanian Plain and Dobrogea, but few cases have been recorded in Transylvania and Moldova.

The cases were disseminated through large area even when their number was small.

Analysis of meteorological data from periods when there were three outbreaks of WNV neurological human infections known in Romania (1955, 1964 and 1996) showed that before each of these three outbreaks was recorded a period with temperatures and precipitations higher than the average values of reference (on '60) for that area. From this analysis it is observed that the higher temperatures and higher rainfalls from the pre-epidemic period were leading to increased mosquito populations. We can say that the main meteorological factors, the temperature and the precipitation are significant in causing outbreaks of WNV infection that led to the emergence of abundant vectors populations.

After the outbreak of 1996 have been analyzed meteorological data for areas and periods where occurred human cases of WNV neurological infections. These data were corroborated with seroprevalence data from the main hosts (wild and domestic birds) and the tangential hosts (horses) in those areas.

Populations of mosquito vectors

Evolution of climatic factors in 2007 had a drastic effect on the mosquitoes' fauna that result a very low abundance of mosquitoes populations throughout the country and the mosquitoes' total lack in certain areas and time periods. Mosquitoes fauna had an extremely low abundance in the Southern half of the country. Mosquitoes populations began to gradually increase in August when rains began to fall in some places.

The mosquitoes study from the Danube Delta and the lagoons area

The mosquitoes studies from the Danube Delta and the lagoons area were performed in the period February - October in Tulcea County (at Mila 26 - the Maliuc area, Sălcioara and Grindul Lupilor). The mosquitoes (5,125 specimens) were collected on humans and on the bird bait trap, by light trap (CDC), entomological net (from sheltering on vegetation places) and by manual vacuum from animal shelters, birds cages and inside of the houses. In November 2007 were collected hibernate mosquitoes from Tulcea.

Culex pipiens was the most dominant species from the mosquito fauna which is unusual in the Danube Delta and the lagoons area. This species usually has a relatively low presence in the Danube Delta, especially in natural habitats (wetlands) that are not suitable for larvae of this species. Because these wetland habitats disappeared (caused by drought) this species was dominant in this year at Grindul Lupilor and Mila 26. *Culex pipiens* larvae have developed into small puddles that remained on dry soil (are actually the specific development habitats).

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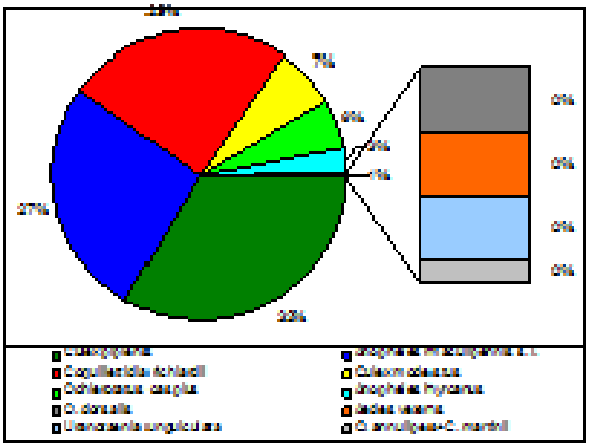

Fig. 1 - The mosquitoes species collected from the Danube Delta and the lagoons area - 2007

In the fig.1 we can see that the mosquitoes from *maculipennis* group were among the dominant species of mosquitoes' fauna in the Danube Delta and the mosquitoes' species from *Anopheles genus* (identified in 2007) were *Anopheles atroparvus* and *Anopheles messeae*.

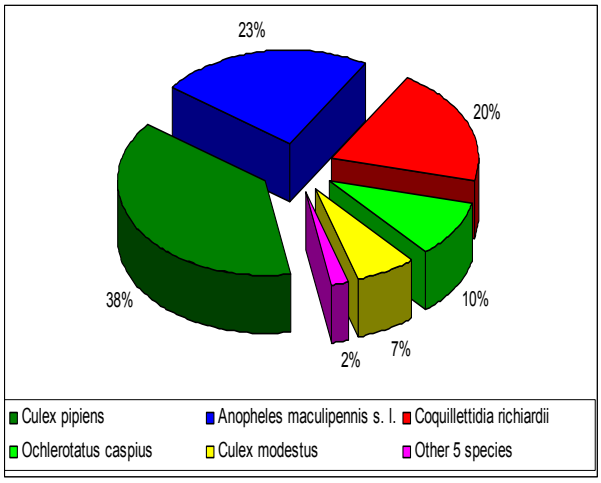

Fig. 2 The mosquitoes species collected from Grindul Lupilor – 2007

From fig. 2 it is observed that the species of the spring genera *Aedes* and *Ochlerotatus* had very low densities up to total absence. Due to specific and unusual climatic conditions, low density of these species was a general feature of the mosquitoes fauna of Romania in the period 2005 – 2007.

The dominant species in the mosquitoes fauna collected from Grindul Lupilor and Mila 26 were the same in 2007: *Culex pipiens, Anopheles maculipennis s.l.* and *Coquillettidia richiardii*. Compared with 2006, *Culex pipiens* was the dominant species in these two areas, instead the *Ochlerotatus caspius* species.

The mosquito species that were collected from Sălcioara: *Coquillettidia richiardii*, species from *maculipennis* group, *Anopheles hyrcanus* and *Culex pipiens* that reached only up to 4%. The habitats of larval development that are located in this area with large reeds channels were not too much affected by drought.

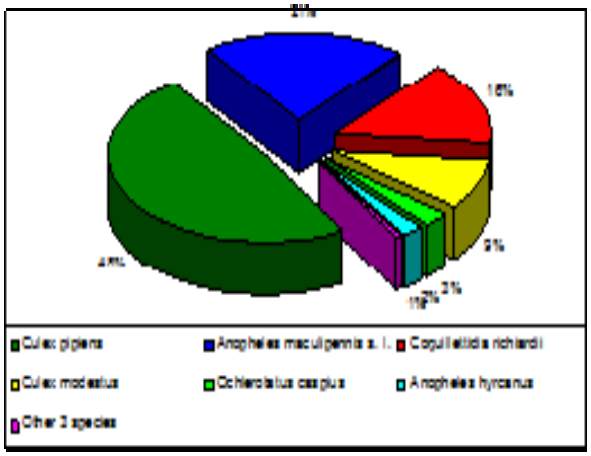

Fig.3 The mosquitos' species collected from Mila 26 - 2007

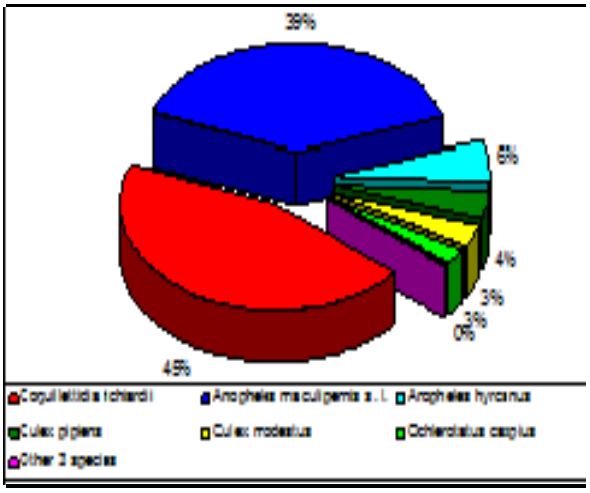

Fig.4 The mosquitos' species collected from Salcioara – 2007

The mosquitoes study from Bucharest Metropolitan Area (BMA)

The mosquitoes' fauna study from BMA showed extremely low abundance until the total absence in many places and moments of 2007. There was detected only one species, *Culex pipiens*, because the drought climate from Southern of Romania.

The mosquitoes (about 8,000 specimens) were collected on humans, by the bird bait traps, by the light traps (CDC) and by traps for collecting the pregnant females from blocks. In the winter 2006-2007 the hibernating mosquitoes were collected from the Giurgiu blocks. In Bucharest, in the winter 2006 - 2007 and in October and November 2007, were not found the hibernating mosquitoes from blocks.

The seroprevalence study in wild and domestic birds - main hosts for WNV

To study the seroprevalence of antibodies to WNV at the main hosts we investigated a total of 34 wild bird

species from 14 localities and domestic birds (chickens and hens, turkeys, ducks, geese) from 86 localities.
- The mean seroprevalence of antibodies to WNV in the wild birds was 14.1% - higher than in 2006 (13.4%) and much lower than in 2005 (27.2%);
- The mean seroprevalence of antibodies to WNV in the domestic birds was similar to the value for the wild birds (14.0%) - higher than in 2006 (11.5%) and 2005 (9.8%) and generally higher compared to years after the epidemic of 1996. Exception was in 2001, when was recorded the highest number of human cases of WNV infection, the seroprevalence was 12.4% (slightly below than in 2007);
- The seroprevalence studies conducted in other counties of Romanian Plain gave interesting results: the mean seroprevalence both in the wild birds and in the domestic birds was higher than in BMA and in Tulcea County.
- The high seroprevalence recorded in the domestic birds from several localities in Teleorman, Prahova and Giurgiu counties (compared to other investigated areas) indicated the existence of enzootic outbreaks with very intense circulation of WNV;
- The highest seroprevalence in 2007 was recorded at ducks and the lowest at chickens and hens (11.9%);
- The average seroprevalence in wild birds in Tulcea county was higher at *Phalacrocorax carbo* (27.3%) and only 12.7% at *Passer domesticus*;
- The higher values of seroprevalence in BMA were as usual at sparrows (*Passer domesticus* and *Passer montanus* - more than 20.0% and lower than in 2005, which was 8.1%) even though the number of birds tested was quite small;
- The seroprevalence in the pigeons was 15.8% - higher than in 2006 (8.0%) in BMA and 14.2% in other counties in the Romanian Plain;
- *Streptopelia decaocto* (15.8%) and *Corvus frugilegus* (20.2%) had significant mean values of seroprevalence in different counties of the Romanian Plain;
- The investigated samples number of *Dendrocopos* major was not too great but every time a significant number of samples were positive.

The seroprevalence study in horses – tangential hosts for West Nile virus (WNV)

In 2007, a total of 2,321 sera from horses were investigated for the seroprevalence of antibodies against WNV.

The sera were collected from: Romanian Plain (Bucharest - urban area, 11 suburban localities, including the rural houses, blocks and villas, 9 villages and other 6 counties); Moldova (3 localities) and Dobrudja (Tulcea county).

The highest seroprevalence was detected in all Tulcea County, especially in localities near the Danube and the lagoons and in other localities in the Danube Delta. The seroprevalence values were lower to the Western of the country. The previous studies had the same result.

Table 1

Seroprevalence of antibodies against WNV in horses - 2007

Study areas	Counties	Localities	No.positive samples / No.investigated samples	Seroprevalence (%)
Bucharest Metropolitan Area – BMA (Romanian Plain)	**Bucharest**	**Urban area**	**22 / 65**	**35.8**
	BMA	11 localities	71 / 484	14.7
	Total	**12**	**93 / 549**	**16.9**
Another 6 counties from Romanian Plain	Teleorman	2	25 / 157	15.9
	Prahova	1	1 / 48	2.1
	Dambovita	2	5 / 100	5.0
	Dolj	1	2 / 100	2.0
	Olt	2	0 / 86	0
	Ialomiţa	1	16 / 100	16.0
	Total	**9**	**49 / 591**	**8.3**
Moldavia	**Galaţi**	**3**	**45 / 150**	**30.0**
Dobrudja	**Tulcea**	**12**	**443 / 1031**	**43.0**
TOTAL 2007		**36 localities**	**630 / 2321**	**27.1**

The highest seroprevalence was detected in Tulcea County, especially in localities situated near to the Danube and lagoons and from the Danube Delta.

At the same time, the seroprevalence was high in BMA (urban area) and Galati County. It is important to note that in these two areas appeared the highest number of human cases after the outbreak of 1996, given that 60.0% of these cases during the outbreak of 1996 were recorded in Bucharest and its suburbs.

The lower seroprevalence was recorded in the other six counties of the Romanian Plain and except the

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Ialomita County and from other localities from Northern and Western of Bucharest.

Land Cover and Land Use maps (LCCS/LU)

To obtain the land cover/ land use maps (LCCS / LU) of Romania were performed the parallel and comparative study of satellite imagery and topographic maps (and / or thematic).

We used the information about the different types of land covers and land use (LCCS / LU) and other changes in their time, compared to present possible habitats of WNV circulation (in the 2 studies areas), in the vectors (mosquitoes), in the main hosts (the wild and domestic birds) and in the tangential hosts (the horses and humans).

We performed comparative studies of land cover in the 2 study areas (BMA and Tulcea County), for 2000 and 2003.

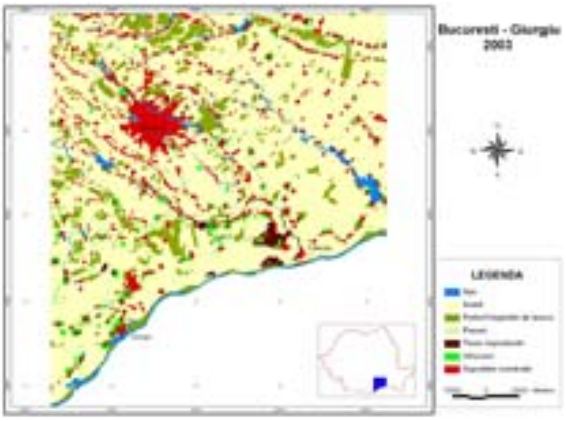

Fig.5 - BMA (Bucharest Metropolitan Area) LCCS / LUCS Map - 2003

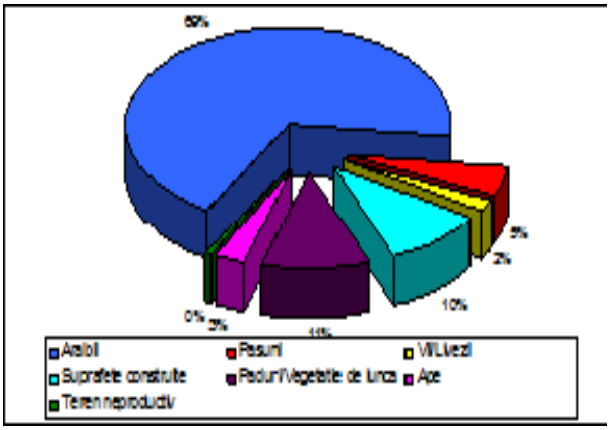

Fig.6 - The land cover/ land use classes' proportions for BMA -2003

Thus, for BMA was found that the surfaces of arable land decreased by two percent and the unproductive land almost halved, while increased the constructed surface, the vegetation areas (forests, meadow vegetation) and the water coated surfaces.

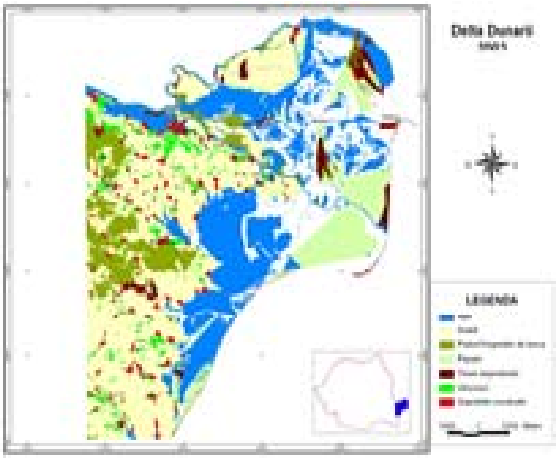

Fig.7 – Tulcea county (Danube Delta and lagoon area) LCCS / LUCS Map - 2003

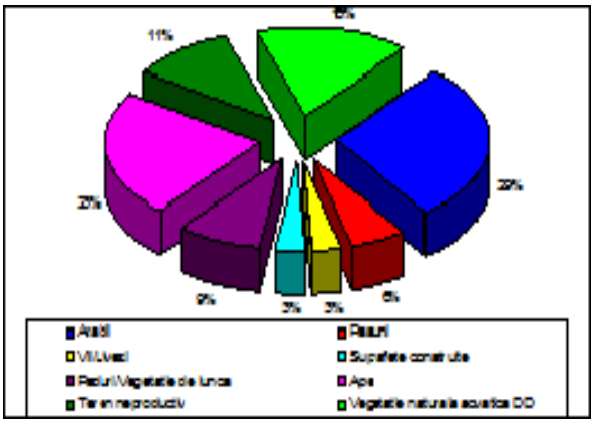

Fig.8 - The land cover/ land use classes' proportions for Tulcea County -2003

During 2000 - 2003, in the Danube Delta and lagoon area decreased the proportion of arable land and the water surfaces and the area of forest-meadow vegetation reduced by one third.

It is important to note that in 2003 the constructed area from lagoons and Danube Delta increased by 50% than the previous size, aquatic natural vegetation areas have expanded by almost 25% and pastures with a 1/5 compared to 2000.

CONCLUSIONS

- The climatic conditions and the habitats influenced the WNV circulation in Romania in different areas and times;
- Special climatic conditions of Romania in 2007 influenced the fauna of mosquitoes' vectors and hosts of WNV;
- The general conclusion of seroprevalence of antibodies against WNV in domestic and wild birds in 2007 seems to be that its values are generally somewhat higher than in 2006 although mosquito populations were extremely abundant (at least until the end of august);

- The high seropositivity wild birds in Romania compared to other parts of Europe show that WNV circulation is extremely intense in Romania. On the other hand, in areas with the lower immunity against WNV birds (eg. U.S.), their mortality is used as a factor in human disease emergence warning (Guptill et al., 2003);

- Our previous investigations have shown that the domestic birds are a good indicator of WNV circulation and their number increasing not only in rural but also in urban areas because they are a permanent presence and spread to rural households type and they live in the areas without blocks in the cities in Romania. They are the main amplifying hosts of the virus in these outbreaks of transmission to humans (Savage et al., 1998, Ceianu et al. 2001). Often the seropositivity rate in the domestic birds' populations in some areas correlates directly with the number of cases of illness in humans in those outbreaks. Therefore, in some time periods or habitats, the domestic birds are a good indicator for the amplification of the virus in different outbreaks, acting as effective sentinels because they are always live in the usual environmental conditions of the area;

- Horses, like the other mammals, including humans, are tangential hosts for WNV. They are a good indicator for the level of the WNV circulation and warning the disease occurrence in humans in a region because they live near the human being and are more expose than these to mosquito bites. Therefore, their seropositivity for WNV is higher than the human population in a certain area, and the seroconversion is most obvious and easily detected in horses. The seroprevalence evaluation of antibodies against WNV in horses highlights the WNV circulation areas and develops the risk territories of transmission in humans;

- The serological investigations on vertebrate hosts of WNV showed that the virus circulation in Romania is intense and takes place on very large areas;

- The mosquitoes' investigations have tried to identify the development larval habitats and the mosquitoes' adults' roosts in natural and anthropogenic ecosystems, the species composition and the ecological characteristics of the main populations in those study areas;

- The investigations in the years following the 1996 epidemic revealed the existence of the main species of mosquitoes in the South of Romania reported in natural and anthropogenic ecosystems (Nicolescu, 1998);

- There was reported the *Culex pipiens* - the dominant permanent specie (primary vector of WNV) both in urban and rural habitats during the epidemic of 1996 and in subsequent years;

- The WNV circulation intensity depends on the quantity of mosquitoes populations, which depend on climatic factors;

- Even in less favorable climatic conditions was recorded the enzootic circulation of WNV on large areas and was demonstrated by the levels of seroprevalence of antibodies to WNV in the main hosts (domestic and wild birds), in the tangential hosts (horses) and in the vectors (species of culicids)

- Our investigations show persistence of WNV circulation in Romania in the context of extending and intensifying its unprecedented global movement (Nicolescu et al., 2003);

- Analysis of land cover/land use maps in 2000 and 2003 showed the changes that appear in the proportions of the classes in the Southern and Eastern of Romania (BMA and Tulcea County);

- Among the significant land cover/land use changes were recorded the decrease of the size of the arable land, the increase of constructed areas and the develop of the pasture areas (from the aquatic natural vegetation areas of the Danube Delta);

- All these changes lead to the emergence of potentially suitable sites for developing and maintaining populations of mosquitoes, vectors for WNV, which requires measures supported by continuous monitoring and control of these populations.

REFERENCES

Asnis DS, Conetta R, Waldman G, Teixeira AA, The West Nile Virus Encephalitis Outbreak in the United States (1999-2000): From Flushing, New York, to Beyond Its Borders. Annals of the New York Academy of Sciences, 951: 161 – 171, 2001

Blitvich BJ, Marlenee NL, Hall R A, Calisher CH, Bowen R A, Roehrig JT, Komar N, Langevin SA, Beaty BJ, Epitope-blocking Enzyme-Linked Immunosorbent Assays for the Detection of serum Antibodies to West Nile Virus in Multiple Avian Species. Journal of Clinical Microbiology, 41, 3: 1041 – 1047, 2003

Ceianu CS, Ungureanu A, Nicolescu G, Cernescu C, Niţescu L, Ţârdei G, Petrescu A, Piţigoi D, Martin D, Ciulacu-Purcărea V, Vladimirescu A, Savage HM, West Nile Virus Surveillance in Romania: 1997 – 2000. Viral Immunology, 14, 3: 251 – 262, 2001

Clarke D H, Casals J, Techniques for haemagglutination and haemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg., 7: 561 – 573, 1958

Dupa G, Duca E, Strat E, Investigations on encephalitis transmitted by arthropods in Moldova County. II. Clinical and epidemiological considerations on six pan-encephalitis cases appeared in spring of 1962 in Roman. Revista Medico-Chirurgicala a Societatii de Medici si Naturalisti din Iasi, 67, 4: 957 – 962, 1963

Ebel GD, Dupuis II AP, Nicholas D, Young D, Maffei J, Kramer LD, Detection by Enzyme-Linked Immunosorbent Assay of Antibodies to West Nile virus in Birds. Emerging Infectious Diseases, 8, 9, 2002

Gubler DJ, Human Arbovirus Infections Worldwide. Annals of the New York Academy of Sciences,

951: 13 – 24, 2001
Guptill SC, Julian KG, Campbell GL, Price SD, Marfin AA, Early-Season Avian Deaths from West Nile Virus as Warnings of Human Infection. Emerging Infectious Diseases, 9, 4, 2003
Hayes CG, West Nile Virus: Uganda, 1937, to New York City, 1999. Annals of the New York Academy of Sciences, 951: 25 – 37, 2001
Miskolczy D, Csiky C, Vendeg V, Abraham A, Waitsuk P, Wagner R, Outbreak of late summer – autumn virus encephalitis in 1955 in Targu Mures (In Romanian). Neurologie, Psihiatrie, Neurochirurgie, 1: 16 – 18, 1956
Miskolczy D, Csiky C, Vendeg V, Abraham A, Waitsuk P, Wagner R, The epidemic of viral encephalitis of the end of summer – autumn in 1955 in Târgu Mureş (the so-called mosquito encephalitis) (in Romanian). Neurologie, Psihiatrie, Neurochirurgie, III, 2: 139 – 147, 1958
Nicolescu G, A general characterization of the mosquito fauna (Diptera: Culicidae) in the epidemic area for
West Nile virus in the South of Romania. European Mosquito Bulletin, 2: 13 – 18, 1998
Nicolescu G, Ungureanu A, Ceianu C, Purcarea-Ciulacu V, Vladimirescu A, Popescu D, Ilina L, The ecology of the West Nile virus in Romania. Bacteriologia, Virusologia, Parazitologia, Epidemiologia, 46, 3: 145, 2001
Nicolescu G, Ungureanu A, Purcarea-Ciulacu V, Popescu D, Ceianu C, Ilina L, Vladimirescu A, Coipan CE, Bordea S, Pitigoi D, Rafila A, Savage HM, Persistence of West Nile virus circulation in Romania after the outbreak in 1996. The 14th European Conference of the Society of Vector Ecology, September 3 – 6, 2003, Bellinzona, Switzerland, OP: 24, 2003
Platonov AE, West Nile Encephalitis in Russia 1999-2001: Were We Ready? Are We Ready? Annals of the New York Academy of Sciences, 951: 102 – 116, 2001
Savage HM, Ceianu C, Nicolescu G, Karabatsos N, Lanciotti R, Vladimirescu A, Laiu L, Ungureanu A, Romanca C, Tsai TF, Entomological and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serological and molecular characterization of a virus isolate from mosquitoes. Am. J. Trop. Med. Hyg., 61: 600 – 611, 1999
Topciu V, Rosiu N, Son C, Pop O, Son Maria, Iana G, Wersching P, Considération sur quelques cas d'encéphalite virotique primaire apparus dans le sud-ouest de la Roumanie (Région du Banat). Archives Roumaines de Pathologie expérimentale et de Microbiologie, 27, 3: 631 – 639, 1968
Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI (for the investigative team), An epidemic of
West Nile encephalitis in South-eastern Romania. Lancet, 325: 767 – 771, 1998