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DETECTION OF ANTIBODIES TO WEST
NILE VIRUS (WNV) IN HUMAN SERA

DETEKCIJA ANTITELA PREMA VIRUSU
WEST NILE U SERUMIMA LJUDI

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*West Nile virus, antibody, test,
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Apstrakt

Virus *West Nile* (WNV) je značajan infektivni agens. Pripada flavivirusima koje prenose komarci i može da ispoljava neurotropizam. Dokazivanje specifičnih antitela u sezoni transmisije komaraca je značajno za identifikaciju rizika za ljude i sezonsku aktivnost virusa. U ovom ispitivanju je testirana prevalenca antitela prema WNV u ljudi koji rade u prirodi i izloženi su ujedima komaraca. Serumima su prikupljeni u periodu od maja do oktobra 2007.godine i testirani na prisustvo anti WNV antitela imunofluorescentnim testom.

Ispitivano je 105 seruma ljudi posle uboda komaraca na prisustvo IgG antitela prema WNV. Od ispitivanih seruma 5 (4.76%) je bilo pozitivno. Antitela su dokazana kod 3 osobe ženskog pola (2.85%) i 2 muškog pola (1.9%). Prisustvo antitela na WNV bilo je češće kod osoba sa više uboda komaraca i u oblastima sa gušćom vegetacijom.

INTRODUCTION

West Nile virus (WNV) is an avian virus that can cause fatal disease in some species of mammals, reptiles and birds. Most clinical cases occur in humans and horses. Approximately 80% of infected humans remain asymptomatic; 20% have flu-like symptoms. Less than 1% develop meningitis, encephalitis or acute paralysis, but some of these cases are fatal or result in permanent impairment (1,2).

Before 1994, disease occurred only sporadically in humans and horses, or as relatively small epidemics in rural areas, and severe neurological signs were uncommon in most outbreaks. Until 1999, West Nile virus was also confined to the Eastern hemisphere. However, severe outbreaks were reported in Algeria, Romania, Morocco, Tunisia, Italy, Russia and Israel between 1994 and 1999, and West Nile virus spread to North America in 1999. An increased incidence of neurological disease and a higher case fatality rate have been associated with these viruses. Some recent viral isolates also cause clinical sign in birds. Consequently, West Nile fever has emerged as a significant human and veterinary health concern in the Americas, Europe, the Mediterranean basin and other areas (3,4).

West Nile virus is an arbovirus in the *Flavivirus* genus of the family *Flaviviridae*. The virus belongs to the Japanese encephalitis virus complex or serogroup.

The flavivirus have characteristics in common: size range 40 to 60nm, symmetry (enveloped, icosahedral nucleocapsid) and type of nucleic acid (positive-sense, single-stranded RNA approx 10,000 to 11,000 bases.

There are at least two genetic lineages of West Nile virus. Lineage 1 viruses contains both virulent and attenuated viruses and have caused most recent outbreaks and is widespread. The strain that entered the United States in 1999, appears to be related to a lineage 1a virus found in Israel from 1997 to 2000 and is among the most virulent strains. Lineage 2 viruses, which occur mainly in Africa, often cause asymptomatic infection or mild disease (5).

West Nile virus was first identified in Africa in 1937, and subsequently, Africa, Europe, Australia, and Asia were recognized as regions in which the virus was endemic

In late summer and early fall of 1999., human West Nile virus (WNV) infections were recognized for the first time in the Western hemisphere. Concurrent outbreaks of encephalitis among crows, humans, and horses in New York State triggered an investigation by human and veterinary health officials that led to the initial detection of WNV in this region.

Since its original introduction into the New York City area, WNV has caused disease in humans, horses, and a wide variety of birds and other vertebrates, spreading into the eastern two thirds of the United States. It is found throughout the western hemisphere, including North America, Central America, and the Caribbean (6,7).

The apparent ability of WNV to be disseminated by infected birds and to persist from year indicated that it will continue to be a public health problem for the foreseeable future (8).

MATERIAL AND METHODS

Between May and October 2007, sera samples were obtained from 105 persons (26 females, 79 males), age range 24-63. Sera was taken from persons working in area with vegetation. All of them were exposed to mosquito bites.

Sera were stored at -20 C until tested.

Serological studies were performed by means of the immunofluorescence method utilizing WNV virus as antigen to detect specific IgG antibodies. The sera samples were defined as positive only when bright green homogeneous fluorescence was seen.

Serological evidence of WNV antibodies was based on the criteria of the test. A positive result was defined as titres IgG of >1:10

Statistical analysis. Data were analyzed using Kolmogorov-Smirnov and χ^2 test.

RESULTS AND DISCUSSION

The prevalence of WNV IgG antibodies was examined using IFA test in 105 sera from person working outside in area with a great vegetation and exposed to mosquito bites.

It was 26 women (24.76%) and 79 men (75.23%).

The presence of IgG antibodies to West Nile virus was found in 5 (4.76%) persons: 3 female and 2 male. There were no significant difference between both groups ($p \geq 0.05$) (Figure 1 and 2). Average WNV positivity was 43.6.

Presence of IgG antibodies was founded in persons with more mosquito bites in 4/26 and with one mosquito bites in 1/79, but there were no significant difference in these groups (Figure 3).

The examined person worked in 3 different areas with high vegetation. There are 33 persons from area 1, 22 persons from area 2 and 50 from area 3. In the area 1 there were the great amounts of vegetation.

Antibody to WNV were found in persons from all areas and there were no significant differences ($p \geq 0.05$) (Figure 4).

The laboratory diagnostic methods used for serologic and virologic diagnosis of WNV are similar for humans and other animal. Serologic testing methods are most commonly used for diagnosis of WNV infection.

Enzyme-linked immunosorbent assays (ELISA) are the most commonly used serological test. Other tests include the plaque reduction neutralization (PRN) test, indirect immunofluorescence (IFA) and hemagglutination inhibition. Rapid tests have recently been developed. In some serological tests, cross-reactions can occur with closely related flaviviruses.

West Nile virus, viral antigens or nucleic acids can be detected in tissues, CSF, blood and other body fluids. Immunohistochemistry to detect viral antigens is mainly used postmortem in cases of fatal neurological disease and PCR test too. Virus isolation requires level 3 biosafety containment, and is rarely performed (5,9).

West Nile virus can be sampled from the environment by the pooling of trapped mosquitoes, testing avian blood samples drawn from wild birds and dog, as well as testing brains of dead birds. Testing of the mosquito samples requires the use of RT-PCR to directly amplify and show the presence of virus in the samples.

Figure 1. WNV positivity in human sera

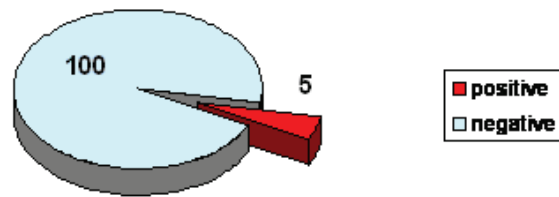


Figure 2. WNV positivity by sex

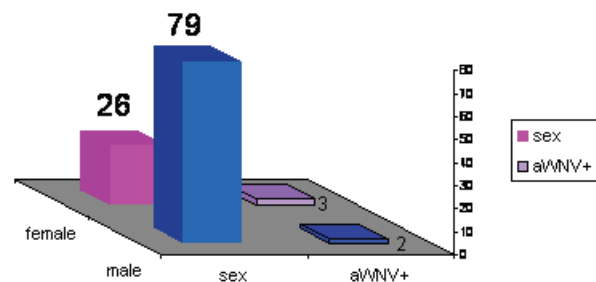


Figure 3. Distribution WNV antibody by mosquito bites

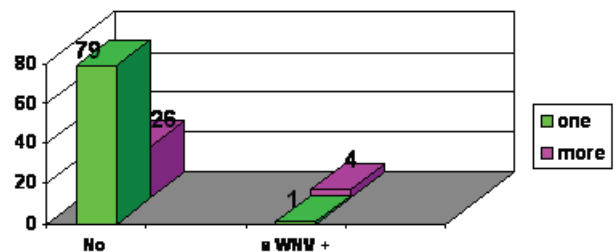
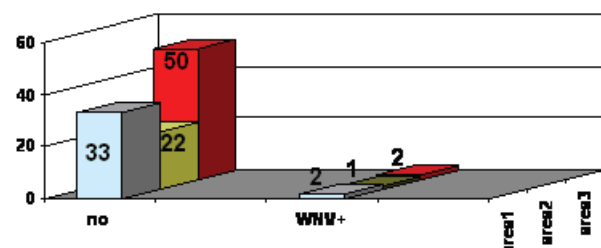


Figure 4. Distribution WNV antibody by areas



The present study was carried out to determine the prevalence of antibodies to WNV in our population. Laboratory diagnosis was established on the basis of an antibody response as detected by serological IFT testing. Currently, the IFA test is one of the methods for the laboratory diagnosis of WNV infection, although its specificity and sensitivity are still questionable. It is generally accepted that this test requires operators with a lot of experience with fluorescence microscopy and that the interpretation of the result is subjective.

In our community 4.76% of 105 persons had an antibody response indicative for WNV past infection.

Antibody to WNV were found in female (2.85%) and in male (1.90%).

Age distribution of presence antibody for WNV showed that antibodies are present in adults of 38-53 ages.

Presence of IgG antibodies was found in persons with more mosquito bites in 4/26 and with one mosquito bite in 1/79.

The examined persons were classified in 3 areas with different amounts of vegetation and the antibody for WNV were found in all areas.

Our first examination about WNV antibody in our population was in 1988 with 174 sera from 89 people who had been bitten by ticks. The sera were taken from some present residents of Belgrade. By use of the haemagglutination inhibition method sera were tested for antibodies to some arboviruses, among them WN. Antibodies to the WN virus were found in 1.12% (10).

There were many examinations about WNV prevalence in human and animal sera in different countries.

In order to estimate the potential WNV threat for Central Europe, the authors from Germany analyzed the anti-WNV prevalence and WNV-RNA incidence among 14,437 and 9,976 blood donors from Germany. There was a high rate of initially anti-WNV reactives (5.9%), but only a few cases were confirmed as anti-WNV positive by neutralization assay. No WNV-RNA positive blood donor was identified in this study. (11).

In 1963-1993, several strains of West Nile virus were isolated from ticks, birds, and mosquitoes in the southern area of European Russia and western Siberia. In the same regions, anti-WNV antibody was found in 0.4-8% of healthy adult donors (12).

In the summer of 1996, southeastern Romania and especially Bucharest experienced an unprecedented epidemic of West Nile encephalitis/meningitis, with at least 393 hospitalized cases and 17 deaths. The surveillance system detected

39 clinical human WN fever cases during the period 1997-2000. Retrospective sampling of domestic fowl in the vicinity of case-patient residences during the years 1997-2000 demonstrated seroprevalence rates of 7.8%-29%. Limited wild bird surveillance data suggest that WN virus persists focally for several years in poorly understood transmission cycles after sporadic introduction or that WNV is introduced into Romania at relatively high rates, and persists seasonally in small foci (13).

The authors from Messina (Italy) examined 1,280 sera during 2006 from 80 stable workers, as jockey and grooms, 100 fowling, 500 blood donors, 600 healthcare workers, 100 veterinary surgeons and 100 hunters in the Messina province to evaluate the prevalence of the WNV infection, by ELISA test, in relation to risk exposure or not. None of the subjects examined has shown positive for antibodies anti WNV (14).

Prevalence of past WNV infections examined by ELISA and confirmed by PRNT in the 504 subjects was 0.6%, affecting mainly older persons (mean age 65), those living in rural areas 5.4% vs. 0% in urban areas and individuals with risk professions (prevalence 2.8% vs. 0%). These results strongly suggest past circulation and exposure of the human population to WNV in southern Spain (15).

The enzootic cycle of WNV involves the transmission of the virus among wild birds and infected *Culex* mosquitoes.

The most important risk factor for WNV infection is exposure to infected mosquitoes. During the 1999 outbreak in New York City, human cases were clustered in areas with large amounts of vegetation, indicating favorable mosquito habitats. A study of an outbreak in Chicago in 2002 revealed that humans with WNV infection were more likely to live in areas with greater amounts of vegetation, older housing, low population density (16,17).

The virus is transmitted through mosquito vectors, which bite and infect birds. The birds are amplifying hosts, developing sufficient viral levels to transmit the infection to other biting infect other birds.

In the early 1990s, epidemiologic pattern changed (18,19).

WNV epidemiology is complex and highly variable between sites and countries. WNV likely will continue to advance throughout the world within the next years and is expected to follow local and regional flyway patterns of viremic birds. Physicians engaged in diagnosing, treating, and reporting WNV infection need greatly knowledge of this emerging infectious disease.

Abstract

It is known that West Nile virus (WNV) is an important pathogen. West Nile virus is a mosquito-borne flavivirus and human neuropathogen. The detection of specific WNV antibodies in the transmission season is a valuable tool to identify human risk and seasonal virus activity.

In this study, the prevalence of specific antibodies to WNV in persons working outside and exposed to mosquito bites was evaluated over a period of May to October 2007. The specimens were tested for WNV IgG antibodies by means of an immunofluorescence test.

Hundred five persons after mosquito bites were tested for presence of specific WNV antibodies (IgG). Of those patients 5 (4.76%) were positive. IgG antibodies to WNV were determined in 3 (2.85%) of females and 2 (1.9%) of males. Presence of IgG antibodies to West Nile virus was higher in people with more mosquito bites and from areas with high vegetation.

REFERENCES

1. Petersen LR, Marfin AA, *West Nile Virus: a primer for the clinician*. Ann Intern Med 2002; 137: 173-179.
2. Sejvar JJ. *The long-term outcomes of human West Nile virus infection*. Clin Infect Dis 2007; 44:1617-1624.
3. Hayes EB, Sejvar JJ, Zaki SR, et al. *Virology, pathology, and clinical manifestations of West Nile virus disease*. Emerg Infect Dis 2005;11(8):1174-1179.
4. Kuljić-Kapulica Nada. *Virusi kao biološko oružje*, U: Biološko oružje, Vojnoizdavački zavod, Beograd, 2003, p105-117.
5. Gubler DJ, Kuno G, Markoff L.: *Flaviviruses*. In: K Nipe DM, Holwey PM (ed.):2007, Fields virology (5. izd.). Philadelphia: Lippincott Williams and Wilkins, a Wolters Kluwer Business,2007, pp 1153-1252.
6. Kramer Ld, Li J, Shi PY. *West Nile virus*. Lancet neurol 2007; 6:171-181.
7. Eidson M, Komar N, Sorhage F, et al. *Crows deaths as a sentinel surveillance system for West Nile virus in the northeastern United States*, 1999, Emerg Infect Dis 2001; 7:615-620.
8. Mostashari F, Buning ML, Kitsutani PT, et al. *Epidemic West Nile Virus during an outbreak of human encephalitis*, New York, 1999:results of a household-based seroepidemiological survey. Lancet 2001; 358:261-264.
9. Beasley DW.2005, *Recent advances in the molecular biology of West Nile virus*. Curr Mol Med 2005;5:835-50
10. Kuljić-Kapulica Nada, Krstić Lj, Dmitrović R, Živanović-Marinković V, Lako B. *Serological examination of some arbo viruses in people after ticks bites (Yugoslavia)*. Abstracts S81. 2nd Symposium on Arboviruses in the Mediterranean Countries, Dubrovnik, 1989.
11. Pfliegerer C, Blumel J, Schmidt M, Roth WK, Houfar MK, Eckert J et al. *West Nile virus and blood product safety in Germany*. J Med Virol 2008; 80(3):557-63.
12. Platonov AE. *West Nile encephalitis in Russia 1999-2001: were we ready? Are we ready?* Ann NY Acad Sci 2001; 951:102-16.
13. Ceianu CS, Ungureanu A, Nicolescu G, Cernescu C, Nitescu L, Tardei G et al. *West Nile surveillance in Romania:1997-2000*. Viral Immunol 2001; 14(3):251-62.
14. Spataro P, Scoglio ME, Di Pietro A, Chirico C, Visalli G, Macri B, et.al. *Seroprevalence study on the diffusion of the West Nile virus among blood donors, healthcare workers, jockeys, grooms and fowlers, veterinary surgeons and hunters in Messina (Italy)*. J Prev Med Hyg 2008; 49(1):22-5.
15. Bernabeu-Wittel M., Ruiz-Perez M, del Toro MD, Aznar J, Muniain A, de Ory F.et al. *West Nile virus past infections in the general population of Southern Spain*. Enferm Infect Microbiol Clin 2007; 25(9):561-5.
16. Hayes EB, Komar N, Nasci RS, et.al. *Epidemiology and transmission dynamics of West Nile virus disease*. Emerg Infect Dis 2005;11:1167-1173.
17. Brownstein JS, Rosen H, Purdy D, et al. *Spatial analysis of West Nile virus: rapid risk assessment of an introduced vector-borne zoonosis*, Vector Borne Zoonotic Dis 2002; 2:157-164.
18. Dauphin G, Zientara S, Zeller H et al. *West Nile:worldwide current situation in animals and humans*. Comp Immunol Microbiol Infect Dis 2004; 27:343-355.
19. Dauphin G, Zintara S. *West Nile virus:recent trends in diagnostics and vaccine development*. Vaccine 2007;25:5563-76.