
West Nile virus infections in Romania after the 2010 outbreak- a retrospective study in human and animal population

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Abstract

In this study the authors have made a retrospective analysis of West Nile virus infection in human and animals after 2010. Few studies have dealt with this arbovirolosis in the last years and there is no analysis to correlate the evolution in animals and humans. To date it is considered that the largest outbreak of West Nile virus (WNV) encephalitis from Europe was registered in 1996 in Romania. Cases of encephalitis with WNV have been reported in our country before first in 1955 in Transylvania, followed by an outbreak in 1964 in Banat. The outbreak from 1996, ended with over 390 confirmed cases of encephalitis in humans the majority being registered in Bucharest and in the southern area of Romania. The epidemiological situation, after the implementation of a surveillance system for WNV in humans until 2010, was characterized by sporadic cases, WNV being reported yearly, except 2002. The infections were reported mostly in the south and south eastern area of the country. The age distribution in human cases recorded a majority in the group age over 65 years due to the senescence of the immune system and the associated diseases. The gender ratio was dominant for men, considering the occupational factor. In 2010 the second large outbreak of WNV was recorded and 47 confirmed cases of WNV infection were registered. After 2010, WNV encephalitis was recorded yearly and the area of detection was confined to eastern area and slowly to the to the whole country. Human cases were registered yearly in areas where the virus was detected in animals by serological screening. Considering the analyzed data, we can state that the main feature of WNV infection is the continuous virus spreading over the territory both in humans and animals. This can be determined by the climatic changes, that allow the surviving and multiplication of the competent vectors in new areas and maybe of the changes in the migratory routes of the birds- main amplifying hosts. Unfortunately, no statistical correlation between human and animal cases could be made. We can state that WNV is continuing to represent a threat for public health and more in depth research has to be made in order to characterize the evolution of the infection in our country and the causes of its endemisation.

Key words: West Nile virus, distribution, human, animal

Introduction

Vector borne diseases represent a major threat and concern to human health all over the world. By far, the most widespread and current infection with an arbovirus, is the infection with West Nile Virus (WNV), which was reported in Romania even before the biggest outbreak from our country (1996), such being reported since 1955 in Transylvania and in 1964 in Banat county. These confirmed outbreaks were followed at an erratic interval (1996) by a sudden recrudescence, developed in the biggest outbreak of WNV infection in Europe, in urban settings, to date, with a high rate of neurological infections. In 1996, between July 15 and October 12, there were identified 393 serologically confirmed or probable patients with WNV infections. Out of them, 352 had meningitis. Affected patients were distributed among 19 districts in the southern, western, central and eastern parts of the country (Sirbu A., et al., 2011). Regarding age, fatality and incidence increase with age, hence, 17 patients older than 50 years died (Tsai TF et al., 1998).

Until the second major outbreak, between 1997 and 2009, the epidemiological status of the country, was characterized by sporadic cases reported on the south side of Romania (Campbell GL., et al 2001), and according to National Institute for Public Health, the confirmed cases with

WNV were reported yearly, except 2002. Also in this year, the surveillance system set in 1997 was extended to a national level (Sirbu A et al., 2011).

In 2010, the epidemiological phenomenon begun on May and ended on November 15th, and materialized with a total of 170 suspected cases with WNV infection reported at the national level. Of these, 52 have been confirmed by laboratory diagnosis, 5 were probable cases, while the rest of them were clearly negative for WNV (Sirbu et al.2011). Among the confirmed cases have been reported four deaths (two deaths in Constanta county, one in Bucharest and the other one, in Mehedinti county) (ECDC, 2010). The distribution per age, show that the median age was 53.4 years (age range: 12-81 years) (Sirbu et al., 2011), while all the deaths were reported in person over 65 years old (ECDC, 2010).

In the 2010 outbreak, the highest incidence was reported in Ialomita county and Constanta county, which are located in the south-eastern part of the country, in the Danube Delta, where are reported most of WNV infection (ECDC, 2010).

If the human cases are reported yearly, the seroconversion in animals is detected only due to serosurveillance assessments, while most of the researching focus on the detection of antibodies or virus in horses, known as dead-end hosts, and birds, who act as amplifying hosts and also as a reservoirs. Once with the human epidemic from 2010 the OIE.int, reported two outbreaks with WNV in horses (five cases) in Braila County and one outbreak (one case) in Constanta County, without any correlation with human cases from that time (OIE.int, 2010).

Synopsis on chronological evolution on WNV infections in Romania

After the second major outbreak, in Romania, the infection with WNV in humans was reported yearly, the dynamic marking an increasing trend until 2016, while in the next year, the human cases have recorded in lower values (CNSCTB, 2011, 2012, 2013, 2014, 2015) (ECDC, 2016, 2017).

In 2011, the National Institute of Public Health reported 11 human cases (ten confirmed and one probable), most of them in Bucharest (five confirmed cases). Additionally, in the same year, two cases were reported in Constanta, one in Galati, both of which are known to be at risk of transmitting WNV. Also, there have been confirmed cases in Iasi and Tulcea. Since the beginning of the season, most cases have been observed in August (six cases), while the others were in September (three cases) and July (two cases).

Age distribution shows that elderly people are more likely to develop WNV infections due to immunological senescence and also due to other conditions affecting the body's defense mechanism, diabetes and cardiovascular disease (Kleinschmidt-DeMasters, BK et al., 2004), while a 2001 study after the outbreak in New York (1999) shows that the incidence of neurological signs is ten times higher in people aged 50 to 59 and 43 times higher in older people aged 80 (Nash D. și col.,2001).

For the same year, the seroconversion induced by WNV in animals was detected in serum samples collected from migratory and resident birds, from Buzau, Braila and Tulcea during their breeding season. The results show that out of 53 serum samples, 20 had antibodies anti-WNV Ig G-type (Pastiu AI et al., 2016).

Presence of specific antibodies anti WNV, were detected in horses from six counties from the eastern side of Romania (Bacau, Braila, Galati, Iasi, Vrancea and Tulcea). In this study, a total of 808 serum samples were collected and tested, and out of all, 473 were Ig G positive (Ludu et al., 2014).

The results obtained from serological screening in animals in seven counties in the eastern part of Romania complete the classic virus transfer route, involving transmission from invertebrates

to vertebrate hosts, that could act as amplifying hosts, (birds) and horses that can not transmit the virus due to low levels of viraemia, and also confirm that horses remain a good indicator of WNV presence (Ludu et al., 2014).

In 2012, the evolution of WNV infections in humans, was marked by a slight increase in cases. According to the National Institute of Public Health, in 2012, 14 suspected cases were reported. Of these, 13 have been confirmed by laboratory diagnosis. The most cases were recorded in August (11 cases), while the rest of them were in September (two cases) and July (one case). The spatial distribution of cases encompasses counties from the south side of Romania (Ialomita, Ilfov, Giurgiu, Braila and Bucharest), but also Iasi county.

The gender ratio between men and women was 1.1, the ratio would be assumed by incriminating the occupational factor. As it was in the previous year, the most affected were the elderly, in 2012 being reported the death of a 87 years old woman.

In animal's populations, the presence of specific antibodies anti-WNV were detected in birds and horses. According to EFSA, the sampling strategies involved the collection of serum from horses in three villages from Constanta and Braila, areas where the Ig M conversion were found in previous year. In June, August and October were collected 45 serum samples from Constanta County and 183 from Braila County. The detection of Ig M was positive in only one sample (from Braila County) out of 228 samples and the rest of them negative. Finding one positive case meant that the viral circulation was still present in that region.

The detection of anti-WNV antibodies in birds (migratory and resident) in the same study conducted by Pastiu AI et al, also confirm that the virus circulates among birds from the Danube Delta region. This area represent an important region of passing, feeding, nesting for migratory birds, and for epidemiological importance, this area might be an important way of introducing the WNV by migratory birds (Prioteasa F.L et al., 2007). In this year, 31 serum samples were collected from the same region, and the seroconversion was positive in seven of these.

In 2013, the epidemiological situation shows a slight increase in human cases with a wider distribution, while the detection of seroconversion in animals proves that WNV persists among the horses in the areas tested in previous years.

Within the national surveillance study implemented after the 1996 epidemic, in 2013 have been reported 24 cases and of these, 22 were positive. As in previous years, the most affected people were the elderly, and according to National Institute of Public Health, there was no death reported. Since the begging of season, most of cases were registered in August (17 cases). In September were six cases and in July, only one reported case. The annual report didn't mention whether patients reported travels in endemic areas, mosquito bites, stagnant water around the dwelling, gardening or poultry raising, although these are potentially risk factors that contribute to upstream epidemiological research.

Eleven counties (out of 42) reported confirmed WNV infection in 2013. The highest incidence was reported from Braila county (four confirmed cases), Ialomita county (three confirmed cases) and Tulcea county (two confirmed out of three reported). Confirmed cases were also reported in Bacau, Bucharest, Constanta, Galati, Iasi, Ilfov, Mures, and Sibiu counties. Confirmed cases in the Mureş and Sibiu counties were reported for the first time and the spatial distribution for the rest of the reported cases (the eastern and southern parts of Romania) confirm the viral circulation along the Danube Delta and its persistence in the areas where most of the WNV infections have been reported over time.

If the epidemiological and virological situation of WNV infection in humans was marked by the increase in confirmed cases, the spread of the virus in new areas (Mures and Sibiu counties)

and its persistence in endemic areas (Constanta, Tulcea, Bucuresti counties), the assessment of the national situation, involved the sampling from areas where Ig M conversion were found in 2011.

Serological surveillance in animals comprised areas from three villages in Constanta (56 serum samples) and Braila counties (25 serum samples). The results obtained by testing serum samples collected from domestic horses in June, August and October show that there was no viral circulation in these areas during the 2013 season (European Food Safety Authority, *Trends And Sources Of Zoonoses And Zoonotic Agents In Humans, Foodstuffs, Animals And Feeding Stuffs*, 2013).

A study by Ludu L. and others in 2013, which included the collection of 110 samples of horse serum from Buzau (ten samples), Braila (40 samples) and Tulcea counties (60 samples), proves the opposite of the results obtained by the monitoring EFSA. The sampled horses didn't have any history regarding epidemiological movement.

Of the 110 samples, 60 were randomized and tested for anti-WNV antibodies (IgG type). Out of the 60 serum samples tested for the presence of anti-WNV IgG, 32 were positive. Seroprevalence in the target areas was 53.3%. IgG was confirmed in 24 of the 32 samples in Tulcea county, in 8 out of 20 samples collected from Braila County and in no evidence from those collected in Buzău (0/8). For the detection of Ig M, from all the positive samples (32), only two were positive for Ig M: one from Tulcea county and the other one from Braila. Considering the seropositivity, it is assumed that the virus circulated in those areas.

The difference in seropositivity obtained in the two studies (EFSA, no positive sample, Ludu et al., two IgM-positive samples, both collected from Braila county) can be attributed to the fact that IgM may be present between three and eight days after the onset of the disease and may persist for up to 30 -90 days, whereas IgG may persist many years (Terrestrial Manual, OIE.int, 2013).

Although mosquitoes, primarily of the genus *Culex*, are the main vectors (Huba'lek Z, et al., 1999) both soft and hard ticks can be infected with WNV, but are unlikely to play a significant role in transmitting the virus (Hayes EB, et al., 2005). However, WNV has been isolated from ixodid and argasid ticks in Europe, placing them secondly after mosquitoes, as well as their importance as vectors (Lawrie CH., Et al. 2004) and their potential to act as a reservoir of the virus (Lawrie CH., et al. 2004). In the same year, in Romania, it was shown that ticks are involved in natural transmission, they can act as a replacement vector, resulting in a bird-tick-bird cycle (Koustiukov MA, et al., 1985). The WNV was detected in an immature tick (nymph), identified as *Hyalomma marginatum marginatum*, collected in Danube Delta Biosphere Reserve, at the end of August 2013, the finding emphasizing the role of ticks in introduction and maintenance of the virus in infections (Kolodziejek J., et al., 2014).

In 2014, within the national surveillance system, have been totally reported 24 human cases, after the onset of the season. Most of the cases were reported in August (14 cases), as it was in previous year, while in September were reported eight cases and in October, two. Cases, though not many, covered the south and southeast side of country, known as being endemic (Ialomita, Giurgiu, Braila, Constanta, Galati, Valcea, Olt, Dolj, Teleorman, Dambovita, Prahova and Bucharest counties), the central (Sibiu and Mures counties) and the northeast (Iasi county) sides of the country, the occurrence of cases in newly areas, showing a wide viral circulation.

In terms of demographics, especially gender ratio and distribution by age groups, in 2014, both the age and the sex ratio, was as in previous years. The average annual incidence of WNV infections, is higher with increasing age (the most affected age was above 65 years old), and the distribution by gender, was higher among males than among females. The associations between increasing age and a higher incidence of WNV infections among males are not clearly, but it might

be related to either reporting biases or the presence of underlying conditions that might be risk factors for the development of the disease (Lindsey NP., et al.2010), due to the occupational factor related to open-air occupations.

The serological investigation in animals shows a continuity of the sample collection of sera from horses from Constanta and Braila counties and the sampling of sera from backyard domestic birds, during a serological evaluation that took place 2011 - 2014.

The strategy involved sampling from 45 Braila County and 161 from Constanta County, and all of them (206 serum samples) were negative for Ig M (EFSA, 2014).

Regarding the serological screening for birds, 21 serum samples were collected from private households in Danube Delta. The samples were from *Gallus gallus domesticus* (n=16) and *Anas platyrhynchos domesticus* (n=5), and tested for detection of Ig G-type antibodies anti-WNV. As a result of testing, seroconversion induced by WNV was observed in both *Anas platyrhynchos domesticus* and *Gallus gallus domesticus* (Pastiu A.I., et al, 2016).

In 2015, there was an increase in diversity in the species studied. Thus, in addition to the collected samples from the horses, during the monitoring program, samples from wild boars were also collected in order to demonstrate the seroconversion and the overcoming of the species barrier of the virus.

The samples from horses (n=171) were collected from the same areas that were targeted in previous years, Braila (n=129) and Constanta county (n=42), and the aim was to demonstrate the viral circulation or its persistence in those areas. Out of these, 12 samples from Braila county were positive for antibodies anti-WNV (Ig M), showing a persistence of virus circulation in this region (EFSA, 2015).

The purpose of this study was the detection of specific antibodies in other species but birds and horses, and to prove that there are many susceptible species to infection, both domestic and wild, more accurate, the author's aim was to assess the seroprevalence of the specific anti WNV Ig G antibodies in wild boars. For this purpose, there were collected serum samples from Bacau (n=30) and Galati (n=38) counties. Out of these, 16 samples from Bacau county and 27 samples from Galati county were positive. These results, prove the circulation of WNV or/and other related flaviviruses among wild boars (Paslaru A., et al, 2015).

In humans, since the beginning of the season, the incidence rate has been 32 cases. Most cases occurred in September (15 cases) and August (13 cases), the rest of cases being recorded in July, October and May. Demographic aspects such as, age distribution, shows that the most affected were elderly, and the gender distribution showed that majority of WNV infections were in male.

The cases have been recorded in the south and southeast sides of Romania (Argeş, Buzău, Brăila, Ialomița, Giurgiu, Constanța, Dolj, Mehedinți, Ilfov and Bucharest Counties), in central and west side of the country (Sibiu, Cluj and Bihor counties) and in the northside (Iasi County) (CNSCTB, 2015).

2016 recorded the highest incidence, since the 2010 outbreak. If in previous years the incidence's rate had a constant value, in 2016, the incidence value was almost three times higher (n=93) than the one recorded in 2015 (n=32), the year with the highest number of cases between 2011 and 2015.

Out of the total number cases with WNV infection, 85 were classified as confirmed, and the rest of them were probable. The cases were recorded from July 1st to October 10th, and the peak of incidence was in August and September.

The spatial distribution of human cases, shows a continuation of the virus's existence in this area, and also confirms its existence in the south and south-east of the country, known as

endemic area. Cases were also recorded in the north side of the country and for the first time, WNV was detected in Satu Mare County (Figure 1).

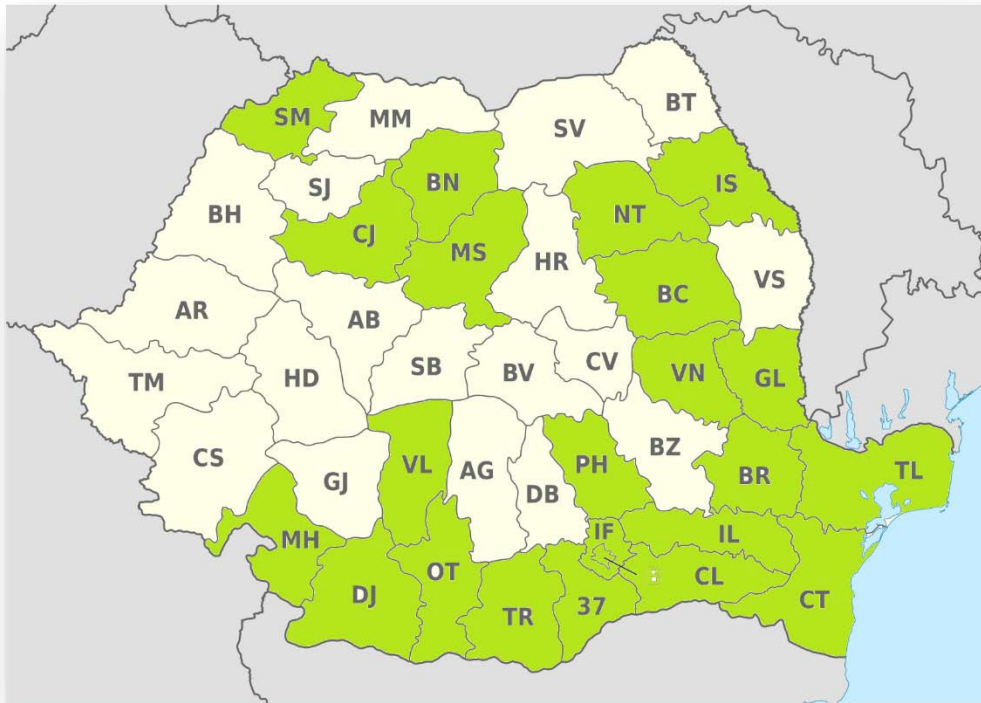


Fig. 1 Spatial distribution of human cases in 2016

The most of cases were recorded in Bucharest (n=18) and in Braila county (n=17). In Bucharest, besides the increased number of confirmed cases, there were registered also the most deaths (n=6), and although the Mures and Teleorman Counties recorded only one cases, in both cases, the patients died (Table 1).

Table 1 The incidence of WNV infections in humans in 2016

County	Confirmed cases	Deaths
Bacau	7	2
Bistrita	1	0
Bucuresti	18	6
Braila	17	1
Calarasi	1	0
Constanta	3	0
Cluj	1	0
Dolj	3	1

Galati	8	0
Giurgiu	4	2
Ialomita	5	2
Iasi	5	1
Ilfov	6	1
Mehedinti	1	0
Mures	1	1
Neamt	1	0
Olt	3	0
Prahova	3	1
Satu Mare	1	0
Teleorman	1	1
Tulcea	2	0
Valcea	1	0
Vrancea	3	0
Total	93	19

Regarding the demographic characteristics, the average age was 61.9 years (maximum 88 years and minimum 15 years). Out of all cases, 51 of them were female patients and the rest of them, were male. According to National Institute for Public Health, there are no differences between sex distributions, this is an exception to the fact that men would be more prone to WNV infections.

All the confirmed cases admit the mosquito's bites outdoors and none of them received blood transfusion or an organs transplant. The risk factors for all the deaths were represented by premorbid conditions like heart diseases, alcohol abuse, chronic renal disease, neurological affections, diabetes, hepatitis B virus and malignancies. However, according to a 2005 study, diabetes, represent a risk factor for developing neuroinvasive disease (Cynthia M.J et. al., 2007), also it is a predictor of severe illness (Patnaik JL et al., 2006). Diabetic patients are more prone to WNV infection, due to diabetes' role in impairing immune status may lead to an increase in the magnitude and duration of viremia (Campell GL., et al., 2002). Out of all the risk factors, alcohol abuse, diabetes and hypertension, were more commonly noted in the medical records in a descriptive study conducted in 2003 (Bode AV., et al., 2006)

In 2016, the antibodies against WNV have been detected in dogs from four counties from the east side of Romania, within a study that involved the collection of samples from areas where in previous years, the infection with WNV was diagnosed in humans and detected in other mammals and birds. Then have been collected 76 blood samples from dogs from Bacau, Suceava, Tulcea and Iasi Counties. As a result of performing the serological screening, seropositive dogs were found in all the counties, showing a wide geographical distribution of this virus in dog populations from households and shelters (Crivei et al., 2016).

In 2017, according to ECDC, there is a slight decrease in human's incidence. If in 2016, there were 93 confirmed cases, in the next year, there were reported 58 confirmed cases and six

deaths. As it was in the previous year, in Bucharest were reported most of cases (n=16) and deaths (n=3), followed by Giurgiu (n=9) and Braila (n=5) Counties.

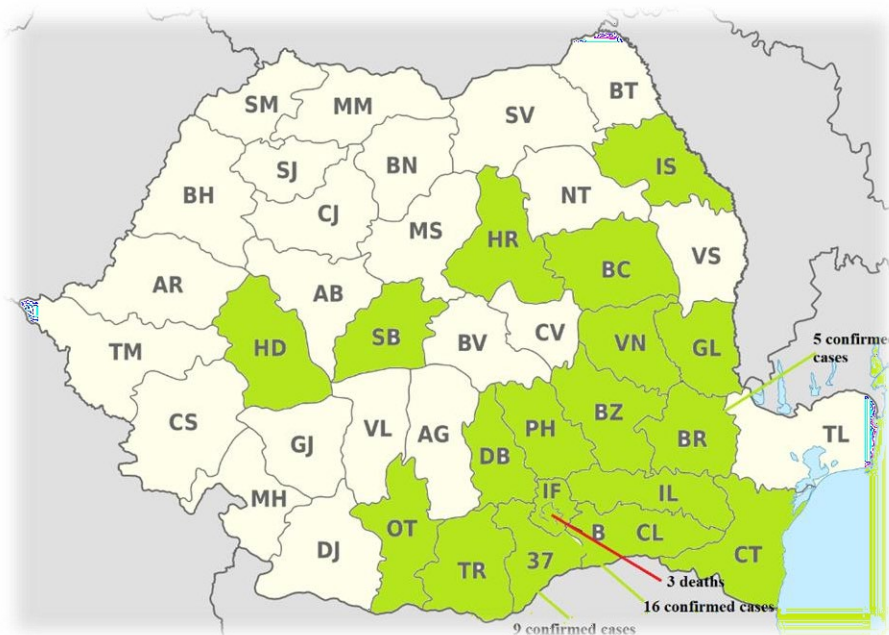


Fig. 2 Spatial distribution of human cases in 2017

Conclusions

WNV is a category B (Bigham AW., 2011) pathogen that debuted as an epidemiological phenomenon in 1955 but had a major impact on public health in 1996. After that outbreak, a national surveillance system was implemented in Romania recording WNV infections. The second major outbreak occurred in 2010, when 57 cases were reported in humans.

After 2010, WNV infections were reported annually, with the evolution trend showing a decrease in the number of cases after this year so that a constant number of cases (11 and 13 cases) could be recorded in 2011 and 2012. In 2013 - 2015, there has been a steady increase in cases, and in 2016, most cases have been reported since the 1996 epidemic (n=96). In the last year surveyed in 2017, a decrease in the number of cases was reported, reported 58 cases.

Spatial distribution of human cases shows a persistent circulation of the virus in the south and south-east sides of the country, known as endemic, but from year to year, it can see the spread of the virus to other parts of the country (the center and the west sides - Sibiu, Mures and Bihor counties). The persistence of viral circulation in the south of the country could be favored by the existence of the Danube Delta, which represents an important area of passing, nesting and feeding for the migrant birds (Prioteasa FL., et al., 2007), and also because of the abundance of the abiotic factors (temperature, rainfall, humidity, the existence of stagnant water) that favors the density and the behavior of the vectors, and also establish the inter-relationships among virus, vectors and vertebrate hosts (Krmer LD., et al., 2016). The spatial distribution of the virus is in line with the European Mosquito Bulletin, according to which, the main vectors of the WNV (*Culex pippiens*) are distributed along the south, southeast, north and central sides of the country (Nicolescu G., et al., 2003), also the high number of cases reported in Bucharest, it might be due to the existence of

another competent vector for West Nile Virus, the *Aedes albopictus* mosquito, who was detected since from 2012 (Ceaianu CS., et al., 2016), confirming also the urban outbreaks.

Regarding demographic aspects, almost in every year, the elderly and the male sex were predictors of developing neuroinvasive disease, but also diabetes, alcohol abuse, heard diseases, hypertension, renal disease appeared to be a significant risk factors for developing WNV infection.

In animals, the WNV infections were detected as a result of the serological screening, the virus being detected in horses, domestic birds, wild boars and dogs. The wide variety of species shows that the virus circulates among animals populations, and also its ability to overcome the species barrier. Also the animals might be used as sentinel for the early detection of the virus in human populations.

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