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MISSION REPORT

West Nile virus infection outbreak in humans in Romania

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JOINT ECDC/WHO REGIONAL OFFICE FOR EUROPE

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The ECDC/WHO Regional Office for Europe joint mission team would like to express their sincere thanks to the Romanian Ministry of Health and the Romanian National Institute of Public Health for the invitation and the subsequent organisational efforts that ensured an efficient and smooth-running mission.

The mission team would also like to thank all Romanian experts who, despite the frequent interruptions to their normal work routine, were always more than willing to share their experiences with, and knowledge of, West Nile virus in Romania.

Note: All epidemiological, virological, entomological and veterinary data in this mission report were obtained from the Romanian National Institute of Public Health, the Cantacuzino Institute, or the Romanian National Veterinary and Food Safety Authority.

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Abbreviations

ADNS	EU Animal Disease Notification System
BSL	Biosafety level
CSF	Cerebrospinal fluid
ECHO virus	Enteric cytopathic human orphan virus
ELISA	Enzyme-linked immunosorbent assay
EWRS	Early Warning and Response System for the European Union
ISCIII	Instituto de Salud Carlos III
NAT	Nucleic acid testing
NRL	National reference laboratory
RKI	Robert-Koch-Institut, Berlin
TBE	Tick-borne encephalitis
WNV	West Nile virus

1 Background

Suspected outbreaks of West Nile virus (WNV) infection have been reported in Romania since the 1950s. Outbreaks of encephalitis, which were serologically confirmed to be caused by WNV infection, were recorded in 1955 in central Transylvania, followed by an outbreak in 1964 in Banat county (central Romania) [1]. The largest outbreak of WNV infection in Europe to date was in Romania when in 1996 over 800 clinical cases of neuroinvasive disease were reported, 393 of which were confirmed for WNV. A total of 17 deaths were reported in this outbreak. The majority of cases were resident (and probably infected) in the capital, Bucharest [1]. Following this outbreak, Romania implemented a surveillance system for WNV infection. The epidemiological situation between 1997 and 2009 was characterised by sporadic cases reported from the southern part of the country (south of the Carpathian Mountains). Data from studies conducted between 1997 and 2000 show that 39 confirmed cases were detected in this area [2]. National surveillance data indicate that between 1997 and 2004 a total of 82 neuro-invasive cases were reported in this area (unpublished data; Romanian National Institute of Public Health).

In 2008, the Romanian National Institute of Public Health reported (through the Early Warning and Response System [EWRS] for the European Union) a case of WNV infection in a 19-year-old male resident of Bucharest, with onset of symptoms in September 2008 [3]. A second case was identified on 22 September 2008 in a resident of Bucharest who was a boatman on a nearby lake. In 2009, also through EWRS, a further case of WNV infection was reported in a 52-year-old male, resident in Dolj county, with disease onset recorded for August 2009 [4]. A second case was identified on 7 September, this time in a 41-year-old man from Prahova county, who had been fishing in the Danube river delta near the village of Jurilovca-Salcioarele (Tulcea county). The onset of symptoms occurred only a short time after his return from the Danube delta.

In 2010, the epidemiological situation appeared to have changed: By 13 October 2010, 47 confirmed cases of WNV infection were reported between July and September 2010, and for the first time in more than ten years, the geographic locations of reported cases included counties in the central and northern part of the country.

This report covers the main findings of the ECDC/WHO Regional Office for Europe joint expert mission to Romania in October 2010.

2 Objectives of visit

2.1 Overall objective

The overall objective of the mission was to review the risk assessment on WNV in Romania and to refine and strengthen the current EU risk assessment for WNV transmission to humans.

2.2 Specific terms of reference

- To review the current epidemiological and virological situation of WNV infection in Romania: characteristics of the epidemic in humans (time, place and person) and all information available from veterinary and entomological surveillance as well as measures for blood safety; and
- To further refine the current risk assessment for WNV transmission to humans, particularly its relevance to the EU.

3 Main findings

3.1 Human epidemiological and virological situation

Romania's National Institute of Public Health issued a first notification of a WNV infection outbreak through EWRS on 30 August 2010. In this first alert, the existence of seven confirmed cases and three probable cases of WNV infection were reported, including two deaths in persons over 60 years of age [5].

According to the current EU case definition, a suspected case of WNV infection in Romania is defined as follows: a person over 15 years of age who presents with fever and meningitis, encephalitis or meningo-encephalitis between May and October and who reports a history of mosquito bites. A probable/confirmed case is defined as a person who meets the clinical and laboratory criteria for probable/confirmed cases. A non-case is a case in which no antibody (IgM) for WNV was detected in the cerebrospinal fluid (CSF) and/or serum.

In August 2010, the case definitions used for WNV infections were modified on two occasions to further enhance surveillance for human cases. On 12 August, after reports of a WNV infection outbreak in Central Macedonia, Greece, the case definition was specific for persons who had returned from Greece or who had visited the Danube delta region. On 30 August, after confirmation of the first human case of WNV infection in Romania in 2010, the suspected case definition was modified to include persons over 15 years of age who presented with fever and meningitis, encephalitis, or meningo-encephalitis between May and October, and for whom a clear CSF sample was obtained. Exposure to mosquito bites was no longer included as a criterion during the enhanced surveillance period in 2010.

All available epidemiological data are presented in an official report from the Romanian National Institute of Public Health, dated 13 October 2010.

3.1.1 Epidemiological overview

Since 13 May 2010, 156 suspected cases of WNV infection were reported at the national level. Of these, 46 cases have been confirmed for WNV infection by laboratory diagnosis. Ninety-six cases were negative for WNV infection, one case continues to be probable, and 13 cases are pending laboratory analysis. Of the 46 confirmed cases, four deaths were reported (two deaths in Constanta county, one in Mehedinti county, and one in Bucharest). The calculated case-fatality ratio in confirmed cases is 8.7%. All deaths were reported in persons over 65 years of age. The aetiology for the majority of the other cases was unspecified, but some were infected with ECHO, Toscana virus and *Mycobacterium tuberculosis*.

Personal characteristics

Information on age distribution for the 47 classified cases (46 confirmed and 1 probable) is shown in Table 1. The highest incidence per 100 000 population was recorded in the group aged 60 to 69 years. The gender ratio between men and women is 1.6 to 1. Cases are divided by residence status (urban/rural, with 10 000 residents as the limit between the two status types). The ratio of rural to urban cases in 2010 was 1.1 to 1.

During the epidemiological investigation, all confirmed cases were interviewed on a series of potential risk factors for WNV infection. Of these, 87% reported mosquito bites, 30% reported stagnant water around their residence, 21% reported gardening as a leisure activity, and 30% reported rearing poultry.

Table 1: Age distribution of reported (confirmed and probable) WNV infection in Romania, 2010 (n=47)

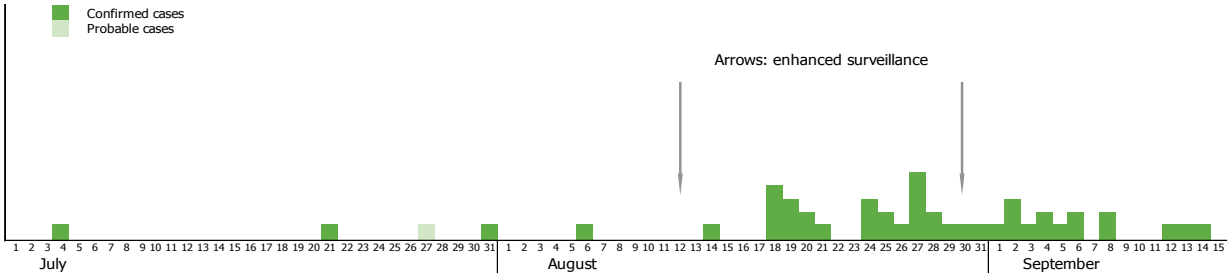
Age group (years)	Number of cases (%)	Incidence per 100 000 population
<20	4(8.5)	0.1
20-29	2 (4.3)	0.1
30-39	7(14.9)	0.2
40-49	4 (8.5)	0.1
50-59	6 (12.8)	0.2
60-69	15 (31.9)	0.8
>70	9 (19.2)	0.4

Source: All data provided by the Romanian National Institute of Public Health

Time

The first confirmed case (retrospectively confirmed) had symptom onset on 4 July and the last on 13 September. The majority of reported cases had disease onset in the month of August (65%), with a peak between the middle and the end of the month. Surveillance was enhanced twice during August (see below), which is indicated on the epidemic curve.

Figure 1: Epidemic curve for confirmed and probable cases of neuroinvasive WNV infection by date of onset, Romania, July–September 2010 (n=47)



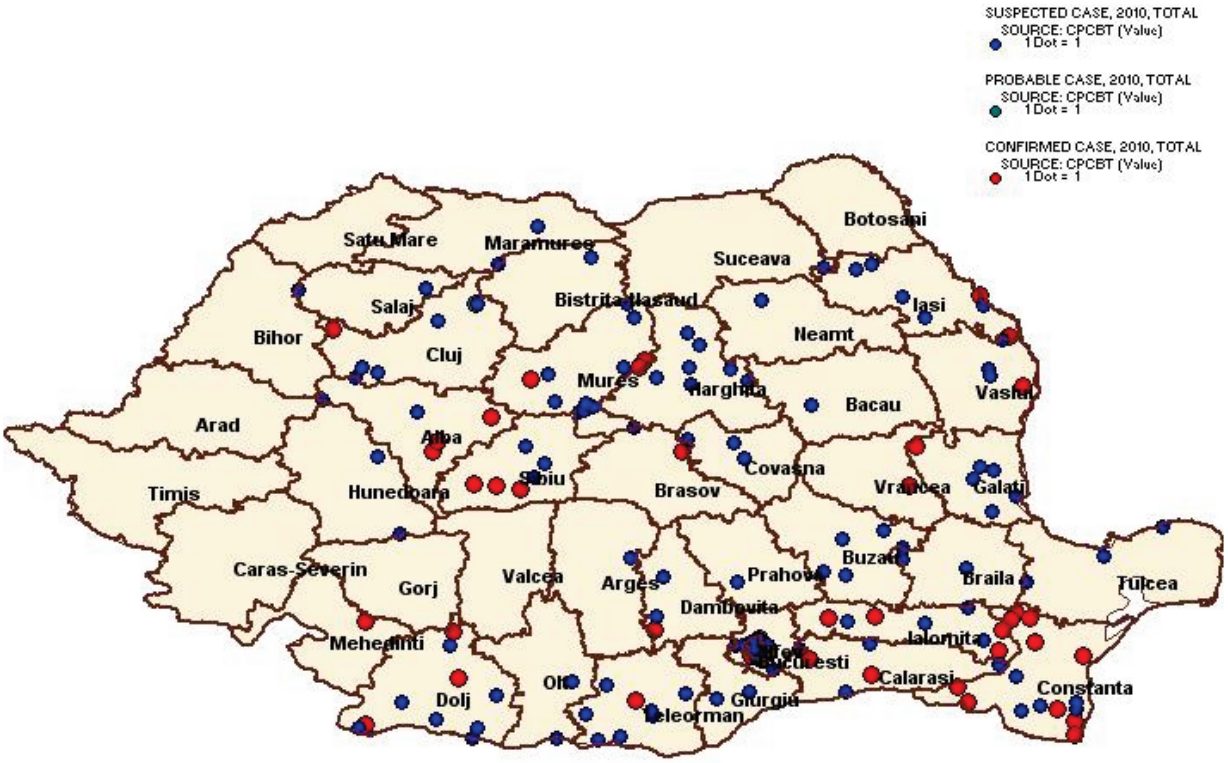
Source: All data provided by the Romanian National Institute of Public Health

Place

Seventeen counties (out of 42) reported confirmed cases of WNV infection in 2010. The highest incidence per 100 000 population was reported from the counties of Constanta and Ialomita (Figure 2 and Table 2), which are located in the south-eastern part of Romania in the Danube delta, which is where historically most WNV infection cases are reported. An incidence rate of over 0.5 per 100 000 was also reported from Calarasi county (close to the Danube delta) and in Alba, Sibiu and Mureş, three counties located in the north-central part of Romania, known as Central Transylvania. In 2010, confirmed cases were also reported for the first time from Iasi and Vaslui counties on the border with Moldova.

In 2010, WNV infection surveillance was implemented nationwide, with 34 counties reporting suspected cases (Figure 2) and sending samples for laboratory diagnosis. Only eight counties did not report any suspected cases of WNV infection during 2010.

Figure 2: Map of suspected, probable and confirmed cases of WNV infection in Romania, July–September 2010



Source: Map provided by the Romanian National Institute of Public Health.

Dot distribution is arbitrary and does not represent the actual address of residence of confirmed, probable and suspected cases.

Figure 3: Map of probable and confirmed cases of WNV infection in Romania, July–September 2010

Source: Map provided by the Romanian National Institute of Public Health.

Dot distribution is arbitrary and does not represent the actual address of residence of probable and confirmed cases.

Table 2: Distribution of probable and confirmed WNV cases in Romania by county of residence and county of exposure (n=47)

	By county of residence	By county of exposure	Incidence rate per 100 000 population by county of exposure
County			
Bucharest	9	8	0.4
Constanta	8	10	1.4
Ialomita	4	4	1.4
Mures	3	3	0.5
Sibiu	3	3	0.7
Alba	3	3	0.8
Dolj	3	3	0.4
Iasi	3	2	0.2
Cluj	2	2	0.3
Calarasi	2	2	0.6
Buzau	1	0	0
Brasov	1	1	0.2
Dambovita	1	1	0.2
Galati	1	1	0.2
Mehedinti	1	1	0.3
Teleorman	1	1	0.2
Vrancea	1	1	0.3
Vaslui	0	1	0.2
TOTAL	47	47	

Source: All data provided by the Romanian National Institute of Public Health.

3.1.2 Surveillance activities

Both the passive and enhanced surveillance systems designed for WNV infection are operated through a hospital-based system. All 42 counties in Romania (41 counties plus Bucharest) have a designated hospital that includes a special ward for infectious diseases or an infectious disease hospital at the county level. All persons presenting with neurological symptoms would be reported to county hospitals for clinical care. Bucharest has two hospitals to which cases of meningitis and encephalitis would be referred.

Passive surveillance

Romania has a national mandatory reporting system for all clinical cases of encephalitis, regardless of aetiology. The specifics of Romania's passive surveillance system for WNV infection are described below.

Romania also operates a specific seasonal (May to October) hospital-based WNV surveillance system, which has been operational since 1996 (after the outbreak in Bucharest). Hospitals report suspected WNV cases – defined as persons that present to healthcare facilities with fever and meningitis, meningoencephalitis and/or encephalitis, are over the age of 15 years, and report a history of mosquito bites – to the county health office. Reporting for suspected WNV infection is compulsory. In addition, they send acute phase serum, CSF, and convalescent serum to the national reference laboratory for WNV serology.

At the EU level, case reporting is done according to the EU case definition (Annex 1).

During the last decade and up to 2008, WNV surveillance was operational in the southern regions of Romania. The counties that participated in this surveillance system included all counties south of the Carpathian Mountains plus the counties in and around the Danube delta.

In 2008, the number of counties participating in the system decreased, when two counties stopped WNV surveillance due to an absence of cases over a period of ten years. In 2009, the system was extended to include all 42 counties in Romania, following the confirmation of two cases of West Nile fever (WNF) in humans in the central part of the country and the detection of WNV-specific IgG antibodies in horses in many other areas of the country. This system has managed to detect outbreaks of meningitis/encephalitis due to other infectious agents such as an ECHO virus type 3 outbreak in 2003 and an ECHO 6 outbreak in 2007. A cluster of meningitis caused by the mumps virus was identified in 2003.

Reporting from the county to the national level for all notifiable disease is conducted on a weekly basis (since 2009). Counties e-mail their surveillance data once a week to the national level where the data are imported into a specially designed Epi-Info database. At present, there are no county-specific outbreak thresholds established for each of the notifiable diseases. For all reported suspected cases of WNV infection, county level epidemiologists carry out a basic epidemiological investigation in which specific exposures and risk factors are assessed.

Enhanced surveillance in 2010 WNV season

In 2010, surveillance for suspected cases of WNV infection was enhanced on two occasions, first on 12 August, when the Greek national authorities reported a confirmed outbreak of WNV infection in Central Macedonia in the northern part of the country [6], and a second time on 30 August when the Romanian outbreak was confirmed. Enhanced surveillance included active case finding around each confirmed case, re-issuing awareness messages to clinicians in all county hospitals on how to recognise the clinical presentation of neuroinvasive WNV infection, and ensuring that reporting and sending of samples was done in a timely manner.

Virological and laboratory activities

Up until 2010, all confirmatory laboratory diagnostics for WNV infection were conducted by the National Reference Laboratory (NRL) for Vector-Borne Diseases within the Cantacuzino Institute. This institute functions independently from the National Institute of Public Health, and the NRL is subcontracted for each clinical sample to be tested for WNV infection. Both laboratories use established testing algorithms for routine WNV diagnostics. The NRL has a maximum capacity of up to 350 samples per week.

In 2010, a laboratory located in Cluj (northern Romania) established the capacity to conduct WNV diagnostics for human samples. The laboratory in Cluj is a public health laboratory and falls under the jurisdiction of the National Institute of Public Health. In August 2010, a proportion of samples from the Cluj laboratory were sent to the NRL for confirmation and quality control. In September, when the Cluj laboratory ran out of reagents for WNV diagnostics, the NRL took over the remaining laboratory analysis functions. Counties in the northern part of Romania send their samples from suspected WNV cases for confirmation to the Cluj laboratory. All counties in the southern part of Romania (including Bucharest) send their samples for confirmation to the NRL.

In terms of diagnostic protocols, clinical samples that are currently processed for WNV diagnosis include acute phase serum, acute phase CSF, and convalescent phase serum samples. In case the patient only presents with West Nile fever and non-neuroinvasive disease, paired samples of sera are used to detect sero-conversion. Routine laboratory diagnostics for WNV rely on serology for antibody detection. For IgM this includes a capture ELISA (commercial, Focus Diagnostics, and in-house) and for IgG an indirect ELISA also from Focus Diagnostics. Comparative tests are also done against antibodies for tick-borne encephalitis (TBE) using IgM capture ELISA (in-house tests) due to the high cross-reactivity of these two infections and the high level of TBE endemicity in parts of Romania. There is some limited capacity in place at the NRL to conduct molecular diagnostics for WNV, including a sensitive TaqMan assay for lineage 1 WNV and a medium-sensitivity SYBR Green real-time assay for lineage 2. There is currently no sero-neutralisation capacity present in Romania, but a BSL-3 facility is currently being constructed at the Cantacuzino Institute, so this capacity might be available in the near future.

Between 10 May and November 2010, the NRL tested 317 samples for 123 suspected cases. The highest number of samples was submitted in the weeks between 30 August and 10 September (64 samples per week). During routine surveillance, testing is carried out by the NRL once a week, with results reported to the requesting clinician and the National Institute of Public Health. During the enhanced surveillance period, testing and reporting was increased to twice weekly. The maximum time for processing at the NRL was three days after the receipt of the sample.

In 2010, a number of samples was sent from the NRL to the ISCIII laboratory in Madrid, Spain, and to RKI in Berlin, Germany, for sero-neutralisation and an attempt at viral isolation and sequencing. To date, no results have been released.

The NRL also conducts laboratory diagnostics for WNV on some entomological, horse and bird samples that are collected as part of ongoing research projects.

3.2 Entomological situation

3.2.1 Historical

Although there is some evidence of potential WNV circulation in Romania in the 1950s, no entomological information from the same period is available.

The large WNV infection outbreak in 1996 was noteworthy for two reasons. Firstly, out of the 15 counties that were reporting cases, the city of Bucharest reported the highest attack rate (12/100 000) compared with other counties (1.5/100 000). Outside of Bucharest, the most affected counties were those located close to the Danube river. In Bucharest, the rate of infection was higher in the more rural areas (28/100 000) when compared with the more urbanised areas (10/100 000) [1; 11]. Entomological surveys were carried out between 1 and 9 October 1996 in Bucharest and Ilfov county, collecting mainly resting mosquitoes with different types of aspirators and some active mosquitoes with different type of traps. Of the 3,689 mosquitoes collected, 94–96% were *Culex pipiens,* and one pool of *Cx. pipiens* resulted in WN antigen positivity [1]. Other mosquito species collected during the survey were *Aedes geniculatus*, *Anopheles maculipennis s.l.*, *An. plumbeus*, *Culiseta annulata*, and *Cx. territans*. Interestingly, no *Cx. modestus* was collected [11]. It should be noted that October is quite late in the mosquito season, so results should be interpreted with caution. It was assumed that it was likely that the *Cx. pipiens* population was abundant in urban areas due to flooded basements (a breeding site not conducive to *Cx. modestus*) and therefore *Cx. pipiens* became the main vector in the 1996 epidemic. However, it is unclear whether *Cx. modestus* may have played some role as a vector in rural areas.

Since 1996, there has been no comprehensive entomological information available.

According to information provided by Cantacuzino Institute (G. Nicolescu), WNV was detected between 2007 and 2009 in *Cx. pipiens* females collected in Bucharest and Tulcea county (including overwintering females). WNV was also detected in *Coquillettidia richiardii*, *Aedes caspius*, *Anopheles maculipennis s.l.,* collected in the same period in Tulcea county. At the time of the team's visit, there was limited information available on the methods used to obtain these results, and on the dates and locations where the collections were conducted as well as the total number of mosquitoes collected (by species) during each collection.

Virus isolations from mosquitoes have been obtained by Cantacuzino Institute on several occasions, and always from *Cx. pipiens*: RO-97 (one strain, Bucharest, October 1996); RO-02 (two strains, Bucharest, August 2002); RO-07 (three strains, near Bucharest, August and December 2007); RO-09 (one strain, rural, November 2009). On some occasions, virus isolation was obtained from overwintering females.

According to a checklist of mosquito species present in Romania [7] and successive distribution studies [8, 9] both *Cx. pipiens* and *Cx. modestus* were present and described over several years: *Cx. pipiens* is abundant in both rural and urban environments, and *Cx. modestus* is mostly found in a rural environment due to its specific ecological needs.

According to information provided by Nicolescu [12], *Cx. pipiens* adult activity in southern Romania usually starts in the second half of May, depending on seasonal climatic conditions, and ends in October: migratory birds arrive early from Africa to the Danube delta, covering the period from March to May, making May the month where *Culex* mosquitoes and migrating birds are in one location at the same time.

3.2.2 Present

According to information gathered by the mission team, the 2010 epidemic shows patterns different from those recorded during the 1996 epidemic. These new patterns may require further investigation (see below for the epidemiological interpretation of the current situation). However, the marked change in the ratio between rural and urban cases reported in 1996 (compared with 2010) is substantial. At present, we cannot say how this different epidemiological pattern is related to the vector population and density in different areas, especially when compared with the outbreak in Bucharest.

3.2.3 Vector control measures implemented

Mosquito control, as well as other DDD measures (disinsection, disinfection, deratisation) are under the auspices of the Romanian municipalities, without a regional/national plan providing guidelines for actions. Due to the specific socio-economic and political situation, each municipality may decide independently if/when/how it activates vector control programmes.

In Constanta, the public health county staff includes two entomologists with relevant experience and knowledge on the vector ecology in the region and access to important historical data that may eventually become useful once re-organised and standardised.

However, during our visit we were not able to meet vector control officers in person and therefore could not gather precise information regarding used products and tools, and the organisation of operations.

3.3 Veterinary situation

3.3.1 Historical

There is historical evidence of viral circulation in both bird (migratory, residential and domestic) and horse populations in Romania. Reports are predominantly from the counties in and around the Danube delta, but also from other parts of the country (personal communication during this mission), which suggests that WNV circulation is common in avian and equine populations in the country. To date, there have been no reports of clinical cases (or deaths) of horses from WNV infection. It is unlikely that high horse mortality rates would go unnoticed. However, it cannot be ruled out that the absence of reports of equine mortality is a surveillance artefact.

Information sharing between the veterinary health and public health authorities has been limited to date. At current, a common legislative order is being developed, which will allow prompt and early information sharing between veterinarian and public health authorities, especially of surveillance data from horses and birds in relation to WNV and avian influenza.

3.3.2 Veterinary surveillance system

At the EU level, all cases of equine encephalitis (regardless of aetiology) are notifiable to the EU Animal Disease Notification System (ADNS). To date, no single clinical equine case of encephalitis has been reported at the national level in Romania, and, for obvious reasons, no single equine case of acute WNV infection has ever been confirmed. However, seroprevalence studies indicate that in many counties infection with WNV is common in horses. In 2010, there was no abnormal bird mortality (for any cause) reported by the animal surveillance system.

In 2009, the veterinary surveillance scheme in Romania was revised, adding a new section dedicated to WNV infection. This new section focuses on passive surveillance for clinically suspected equine cases of WNV infection. It also includes a component dedicated to conducting seroprevalence studies for anti-WNV IgG in sheep, horses, and domestic poultry populations in all counties (results below). All 42 county laboratories have recently been trained in anti-WNV IgG detection, but resource restrictions prevent some laboratories from being fully functional. The National Veterinary Laboratory participated in the quality control scheme in place at the EU level through the European Community Reference Laboratory.

Following the identification of a single or several IgG positive horses, a circle with a radius of 25 km is drawn around the location of the afflicted animal/s and designated as a 'risk zone'. In these risk zones, all horses are tested monthly for anti-WNV IgM from May through October, to establish recent viral circulation. All IgM-positive horses are subsequently tested by PCR for viral identification. In these risk zones, samples from birds will also be tested for the presence of WNV antibodies, using the established surveillance mechanisms for avian influenza H5N1.

Further improvements are envisioned for the surveillance system in 2011. This includes raising awareness among the public for reporting suspected clinical infection of horses with WNV and the introduction of limited entomological surveillance activities. In addition, components of the surveillance system already in place will be further refined.

Veterinary studies results from 2009 and 2010

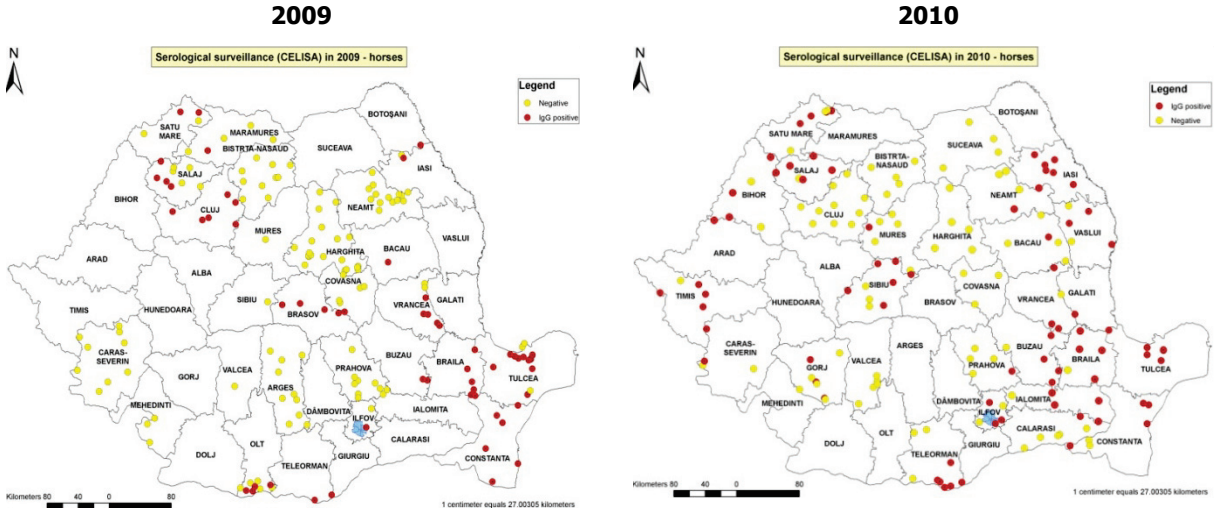
Data from the serological studies carried out in sheep, horses and domestic poultry are shown in Table 3. Figure 3 shows the location and number of IgG-positive horses detected in the seroprevalence studies in 2009 and 2010. These data indicate that seroprevalence for WNV in horse populations in Romania is very high. Furthermore, the geographic distribution of seropositive horses throughout the country suggests that viral circulation for WNV occurs throughout the country.

Table 3: Seroprevalence of anti-WNV IgG in horses, sheep and poultry, Romania, 2009–2010

	2009		2010	
Animals	Animals tested	Animals IgG positive (%)	Animals tested	Animals IgG positive (%)
Horses	1409	471 (33.4%)	1446	357 (24.7%)
Sheep	210	18 (8.6%)	57	0 (0%)
Poultry	304	41 (13.5%)	182	15 (8.2%)

Source: All data provided by the Romanian National Institute of Public Health.

Figure 3: Seronegative and seropositve horses by anti-WNV IgG, included in WNV surveillance 2009 and 2010



Source: All data provided by the Romanian National Institute of Public Health.

Dot distribution on the above map is arbitrary and does not represent the actual geographical location of the tested animals.

3.4 Impact on blood transfusion

At present, Romania does not have nucleic acid testing (NAT) facilities available for the screening of viral infections in blood donations. It is currently under discussion to have such systems in place in time for the 2011 WNV transmission season. Blood safety measures in relation to WNV infection therefore rely on screening of donors and deferral policies for affected areas. Organ transplants are a rare event in Romania.

In the 2010 transmission season, the Romanian National Transfusion Institute issued the first guidance for blood safety measures in the country on 3 September 2010, in light of the evolving epidemiological situation after the first WNV infection case was confirmed on 30 August 2010. The detailed guidance is shown in Annex 2. The following control measures were implemented in 2010:

- Enhanced screening of potential donors, specifically with regard to clinical criteria.
- In affected areas (any county with a case; or in Bucharest, any neighbourhood with a case), donors are called back five days after donation to check if they developed symptoms indicating WNV infection.
- Donors are asked to actively report any fever-like symptoms, up to 15 days after donation.
- Deferral of donors in case of travel to affected areas/countries: Greece, Russia, Turkey, Bulgaria.
- Deferral of donors from communities with cases up until 1 December 2010.
- Deferral of donors in large cities: only donors from the same neighbourhood as the cases are deferred in order to maintain a sufficient blood supply.

In Bucharest, the neighbourhood which first reported a positive WNV-infected case was deferred from blood donation. The result of this decision was a 30% reduction in available blood supplies for Bucharest. Therefore no further deferrals for Bucharest were implemented.

With regard to the implementation of the EU Blood Safety Directive (in particular Annex III) [13] in the event of a WNV infection outbreak, Romania faces clear challenges:

- During rapidly evolving outbreaks it is operationally challenging to produce daily updates for blood collection centres regarding newly affected areas. The logistics of making this updated information available in a timely manner puts a heavy strain on the system.
- The current Directive has no clear criteria on how to define the geographic boundaries of an affected area. This may lead to the deferral of a large number of donors, particularly in larger cities. The impact on the availability of blood and blood products should be evaluated for different scenarios of deferral.

3.5 Other public health measures

In addition to the surveillance strategies implemented for human cases, horses and birds, and the measures implemented to ensure blood safety, further public health measures were implemented during the 2010 outbreak:

- Active case finding around reported cases to identify new cases.
- Communication and education:
  - Local city administrators were informed about the cases and the necessary measures for mosquitoe control.
  - Advice was given to the general population on how to protect themselves from mosquito bites.
- Information sharing between veterinary and public health authorities:
  - Regular reports on the epidemiological situation were shared at county level in Constanta.
  - At county level in Constanta, medical doctors were informed when IgG-positive horses were identified in their area in 2010.

3.5.1 Risk communication and public education

The Romanian Ministry of Health has been issuing information material and health education messages for the public through their website.

4 Risk assessment for Europe

4.1 The importance of the current event

The 2010 WNV infection outbreak in Romania is noteworthy for several reasons:

Size and geographical extension of the outbreak

In 2010, Romania reported the highest number of humans infected with WNV since the large outbreak in Bucharest in 1996. Cases in 2010 were not only restricted to the traditional viral circulation areas around the Danube delta, but were widely spread throughout the country, including central Transylvania, the border with Moldova, and areas with altitudes of up to 600m. It therefore appears that there was a likely extension of the area of viral circulation in 2010 and that the increase in cases in 2010 and the geographic extension cannot be explained by recent changes in the surveillance system alone.

Timing of outbreak and similarity to WNV infection outbreak in Central Macedonia, Greece

When looking at the onset of symptoms of the first confirmed case in July 2010, the similarities to the outbreak of WNV infection in Central Macedonia are striking: both outbreaks showed the peak of the outbreak towards the end of August, eventually tailing off in September. This suggests that the conditions in 2010 were equally favourable to the circulation of WNV in Greece and Romania. Whether these conditions are due to climatic factors (rainfall, temperature, humidity, etc.), the lineage of virus introduced, bird migration, mosquito vectors involved or some other factor is not yet known.

With regard to the climactic conditions, some further in-depth analysis of the situation throughout the Mediterranean and Russia might provide some indications on what was different in 2010 compared to previous years. Unfortunately, there is very limited information available on bird migratory routes that could further elucidate this aspect. No detailed information exists on the implicated vectors in the 2010 outbreak in Romania. However, the equal ratio between urban and rural cases in 2010 indicates that urbanised areas such as Bucharest are now less favourable to the virus when compared with 1996.

To date, no viral isolate has been obtained, so there is no information on the lineage of the circulating virus. A genetic characterisation of the circulating virus would be useful, as modifications of the virus may influence the efficiency of transmission.

4.2 Risk for sustained transmission in Romania

The ecological situation in Romania (presence of resident and migratory birds and competent primary vectors and bridge vectors) favours the successful transmission of WNV. Viral transmission in Romania has been documented every season over many years but with only limited transmission to humans, with the exception of the 1996 outbreak. Therefore it is highly likely that viral circulation will be sustained, either because the virus is maintained in local natural enzootic cycles and/or because of continuous introduction of the virus by migratory birds.

4.3 Risk for the remainder of Europe

Considering the outbreak of WNV infection in Central Macedonia (Greece) and Romania in 2010, it is clear that ecological parameters in parts of Europe are currently favourable for viral activity. Further support for this assumption comes from reports of human outbreaks during the same time in Russia and Hungary, and equine outbreaks in Morocco. This reinforces the need for strengthened surveillance as well as preparedness plans that cover all aspects involved in the transmission of this disease, including human surveillance, entomological surveillance, veterinary surveillance (including birds and horses), and blood and tissue safety.

5 Conclusions

WNV infection in Romania is endemic in bird, horse and human populations. This is evidenced by high seroprevalence rates in horses and sporadic reports of human cases on an annual basis. Viral circulation was intense during a large outbreak in 1996 in Bucharest and it appeared intense and more widespread during the 2010 outbreak. The epidemiological picture seen in Romania in 2010 is similar to the one seen in other countries during the same time period, for example in Russia and Greece. However, other countries in Europe, for example France, which has a history of WNV circulation, did not observe anything unusual this year. These findings further stress the complexity of the WNV epidemiology.

The precise reasons for the intense and widespread WNV circulation in the Mediterranean this year are unclear and probably multifactorial. It is likely that there is some contribution of local climatic conditions to be more favourable for viral transmission from bird populations to humans. However, it is also possible that viral characteristics have played a role. For this reason, it would be important to genetically characterise the virus and try to understand what (if any) influence its genetic makeup may have had on improving vector competence and capacity.

Furthermore, the absence of clinical syndromes in horses despite high infection rates needs to be confirmed. If confirmed, it would be of interest to further explore this and perhaps link it to viral characteristics or characteristics of the horse population.

The joint ECDC/WHO Regional Office for Europe mission has also managed to confirm Romania's extensive experience with WNV. Furthermore, it is evident that there is a wealth of historical and recent data from all sectors (veterinary, human, virological, and entomological). This presents a great opportunity for all involved parties to collaborate and analyse the data using a multi-sectoral approach.

6 Next steps and considerations

6.1 Further steps and considerations: Romania

General aspects

- Further strengthening of the communication of the sectors involved in preparedness and response activities for WNV at national and county level, including the definition of roles and responsibilities before, during, and after WNV infection outbreaks.
- Further strengthening of informal collaboration between all involved sectors and continue to work on further improving the existing mechanisms for the formal exchange of information and technical specifics in relation to WNV.
- Development of a more detailed and integrated cross-sectoral WNV preparedness plan (roles and responsibilities: coordination, command, control, and communication) that also includes operational manuals and standard operating procedures. This can be done by looking at existing multisectoral preparedness plans in Romania (e.g. for avian influenza) and looking at what other countries in the region have in place.
- Creation of an inter-ministerial task force for WNV (ministries of health and agriculture) to update the current strategy and develop a common integrated preparedness plan detailing roles and responsibilities of all sectors involved in a WNV outbreak. This should include the revision of local plans where necessary and the production of a detailed operational manual/standard operating procedures.
- Development of a simple tool which may contribute to enhanced coordination between the different partners, for example the establishment of a 'West Nile Virus Bulletin' to which all organisations and experts involved in the management of WNV-related problems could contribute by providing data and support. The distribution of the Bulletin online would make it accessible to a large number of stakeholders.

Human surveillance

- Maintain surveillance for the coming years in all counties in Romania. Continue to monitor participation of counties in surveillance.
- Maintain the laboratory capacity for WNV diagnostics and ensure that an external quality control system is in place.
- Carry out a thorough analysis of epidemiological data for precise residential condition, professional and leisure activities (consider GIS technology).
- Carry out a comparison of epidemiological data, comparing the 1996 outbreak with the one from 2010 in order to understand the relative magnitude of each outbreak. Such a comparison should also consider the shift in dates of disease onset: in 2010 a higher proportion of cases had dates of onset in September, as opposed to 1996, when dates of onset were mostly in August.
- Identify possible hotspots through a spatio-temporal analysis of human cases over the last 20 years.

Entomology

- Initiate an inventory of the mosquito control activities conducted by various public and private bodies such as Bucharest airport, Bucharest municipality, Constanta municipality and others. This may help in the coordination of vector control activities, optimisation of actions, quality control, and cost/benefit analysis.
- Organise a systematic approach to the collection of entomological information (including the collection of all currently available data) and systemise the analysis with the aim to develop a more complete picture of vector species, seasonality, distribution, and abundance variability per year. Use this information to further understand the WNV incidence in human and horse populations.

Veterinary aspects

- Continue the collaboration established with the European Community Reference Laboratory in Maisons-Alfort, France.
- Ensure that external quality control systems are in place for all laboratories conducting WNV serology.
- Strengthen surveillance of equine encephalitis by raising awareness among horse owners and veterinarians, and by identifying possible obstacles which hinder reporting.

Blood safety

- Continue efforts to evaluate whether resources and capacity are in place to implement NAT screening for blood supplies.
- Re-assess feasibility for continued updating of 'affected areas' list for blood donation exclusion. Logistic considerations of making this information rapidly available should be addressed before the next transmission season: identify communication procedures.
- Create a technical working group with public health surveillance epidemiologists and the national blood authorities to determine ahead of the transmission season what control measures can be put in place and what criteria should be used to implement these measures. These definitions would need to include the concepts of acceptable risk, geographic limits of an affected area, duration of identifying an area as 'affected', inclusion/exclusion of equine cases when classifying an area as 'affected', etc.
- Further elucidate whether there is a role for ECDC in trying to harmonise the answers to these questions in different EU countries.

Risk communication

- There is room for improvement with regard to public awareness of WNV infection and the uptake of good practices that need to be adopted in the event of an outbreak. Current information materials and messages should be reviewed to ensure there is a better understanding of the need for prevention and containment measures.
- It is important to establish good relations with the media from the outset in order to make sure that an outbreak situation is presented correctly and key messages to the public are conveyed quickly and accurately. Well informed media can help the cause by ensuring that the public and the international community are aware of ongoing efforts and by conveying key messages.
- Individual specialists (veterinary and human health) who may need to work with the media should establish communication links before the start of the transmission season. These specialists should receive media handling training, if they have not already taken such a course previously.

6.2 Further steps and considerations: Europe

- Conduct a more comprehensive comparative study, which should include an analysis of climatic conditions, bird species composition, ecological conditions in the virus circulation areas, in order to improve our understanding of the phenomenon; this could also include linking up with existing WNV research projects such as the European Distance and E-Learning Network (EDEN).
- Conduct a thorough review of existing preparedness and response plans for WNV.
- Strengthen existing preparedness plans in countries potentially affected by WNV, and encourage countries that do not have such plans in place to consider their development. Such plans would need to include: relevant public health measures (raising the awareness of healthcare providers and the public regarding issues related to WNV disease management and prevention, including personal protection against mosquito bites); enhancing disease-specific human, veterinary and entomological surveillance; strengthening vector control and monitoring; and addressing blood and organ safety issues to further reduce the already low risk of transmission from viraemic blood and solid organ transplantation.
- Continue to closely monitor the activity of WNV circulation throughout the region.
- Review WNV epidemiology and outbreaks in different countries in Europe to better understand the causes of the current WNV circulation.
- Review the impact of current vector control strategies to prevent and control WNV outbreaks.
- Identify experts that can provide further support.

7 Composition of the ECDC/WHO Regional Office for Europe team

- Henriette de Valk, head of Zoonosis and Emerging Diseases Department, INVS, Paris, France.
- Romeo Bellini, medical entomologist, Centro Agricoltura Ambiente 'G. Nicoli', Emilia-Romagna Region, Italy.
- Ana Paula Coutinho, Alert and Response Operations Programme, WHO Regional Office for Europe, Copenhagen, Denmark.
- Annick Lenglet, Preparedness and Response Support Unit (PRU), ECDC, Stockholm, Sweden.

8 Schedule of visit

Day	Activities
Monday , 11 October, 2010	• Meeting at the National Institute of Public Health: epidemiologists at national and regional level • Meeting at Cantacuzino Institute: national reference laboratory; entomological, ornithological and veterinarian research experience with regard to WNV • Meeting with National Veterinary and Food Safety Authority • Meeting with National Transfusion Centre
Tuesday, 12 October 2010	• County level visit in Constanta to explore all aspects surrounding WNV preparedness and response: entomology and aspects of human and animal health • Meeting with staff from the National Institute of Public Health in Bucharest to discuss the development of WNV risk assessment tool
Wednesday, 13 October 2010	• Debriefing at National Institute of Public Health and further discussion with all involved stakeholders.

9 Persons met

Romanian Ministry of Health:

- Geza Molnar, Amalia Canton

Romanian National Institute of Public Health:

- Adriana Pistol, Florin Popovici, Roxana Serban, Anca Sarbu, Radu Cucuiu, Daniela Pitigoi

National Institute for Research and Development in Microbiology and Immunology 'Cantacuzino':

- Daniela Badescu, Cornelia Ceianu, Raluca Gatej, Silvia Enache, Gabriela Nicolescu, Dr Alexandru Vladimirescu

National Veterinary and Food Safety Authority:

- Hristescu Doru, Ioana Neghirla, Claudiu Diaconu, Florica Brucher, Elena Popa, Minera Burleanu

National Transfusion Institute:

- Corina Posea, Andy Rosin, Mihai Pecec, Doina Gosa

Constanta County Public Health Direction:

- Constantin Dina, Mihaela Dinisov, Suzan Ibram, Eleonora Mihalcea, Camelia Cristu, Leonid Carpus, Marian Ruta

Constanta Veterinary Laboratory:

- Ecaterina Tanase

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Annex 1. EU case definition for human WNV infection

Clinical criteria

Any person with fever OR at least one of the following two:

- encephalitis;
- meningitis.

Laboratory criteria

- Laboratory test for case confirmation (at least one of the following four):
  - isolation of WNV from blood or CSF;
  - detection of WNV nucleic acid in blood or CSF;
  - WNV specific antibody response (IgM) in CSF; or
  - WNV IgM high titre AND detection of WNV IgG, AND confirmation by neutralisation.
- Laboratory test for a probable case:
  - WNV specific antibody response in serum.

Laboratory results need to be interpreted according to flavivirus vaccination status.

Epidemiological criteria

At least one of the following two epidemiological links:

- animal-to-human transmission (residing, having visited or having been exposed to mosquito bites in an area where WNV is endemic in horses or birds); or
- human-to-human transmission (vertical transmission, blood transfusion, transplants).

Case classification

A. Possible case:

N/A

B. Probable case:

Any person meeting the clinical criteria AND with at least one of the following two:

- an epidemiological link; or
- a laboratory test for a probable case.

C. Confirmed case:

Any person meeting the laboratory criteria for case confirmation.

Annex 2. Measures implemented by the Romanian National Transfusion Centre

Precautionary measures for blood safety

Action Plan to Provide Transfusion Safety in the Context of Appearance of West Nile Virus Infection

Ministry of Health, competent authority in the field of blood transfusion, together with the management of National Institute of Transfusion Hematology (NITH) and national experts in blood transfusion, have developed the following plan of action with immediate effect, in order to prevent West Nile virus transmission through blood and blood components:

1. NITH, on the basis of the information received from the Ministry of Health, Public Health Directorate and Control in Public Health will send to the blood transfusion centres the situation of the confirmed human cases of infection with WN and localities where they have been identified.

Blood transfusion centers will also receive from the local Public Health Authority relevant information on epidemiology, clinical and laboratory aspects of WN virus infection, and general measures to prevent transmission of WN virus infection in humans.

2. Blood transfusion centres will apply starting with September 3rd 2010 the following measures:
2.1. For the selection of blood donor and blood components:

a) The physician responsible with donor's selection will perform a thorough clinical examination, and will temporarily defer potential donors showing specific symptoms or signs of WN virus infection and/or mosquito bites.

b) The following additional questions will be introduced in the questionnaire and in the interview:
- Have you travelled to these places in the last 28 days.... ? The localities will be mentioned in Annex 1.
- Have you been fishing or for a picnic in areas populated by mosquitoes? (ponds, lakes, delta)
- Is the basement of the house / block of flats where you live flooded ?

The donor will be deferred for 28 days from donation if an affirmative answer to any of these questions is given. Potential donors who spent at least one night in areas where human cases of WN virus infection were identified, will be excluded from donation for a period of 28 days from the moment of leaving the area. A list of affected areas is contained in Annex 1.

c) The donor will be required to inform the Blood Transfusion Centre if, within a period of 15 days after donation, he/she found the appearance of a febrile episode / - skin rash.

d) Temporary deferral of potential donors living in the affected areas, until November 30th, 2010

e) No mobile collections will be held in the affected areas / localities mentioned in Annex 1, untill November, 30th, 2010
2.2. Formal addresses will be sent by the BE to hospitals where blood components are distributed in order to communicate that:
a) it is recommended to limit the consumption of blood and blood components to emergencies and justified cases until November 30th, 2010.
b) must notify the Blood Transfusion Centre through the hemovigilence network any cases of transmission through blood and blood components of WN virus infection.

3. Blood transfusion centres in counties (districts) without identified cases will take all necessary measures to increase the collection, to compensate the potential shortage of blood components in the affected areas.

4. Ministry of health and NITH will permanently monitor the impact of the taken measures on the availability of blood components and take appropriate action in case it will be affected.

Annex 1

A List of districts in Romania, where human cases of West Nile virus infection have been identified :

Dolj – 1 case

Alba – 1 case

Buzău – 1 case

Cluj- 1 case

Ialomita -4 cases

Galati- 1 case

Sibiu- 2 cases

Municipiul Bucuresti - 7 cases

Teleorman – 1 case

Mures - 2 cases

Constanţa - 7 cases

Călărasi – 1 case

Iasi - 3 cases

Vrancea- 1 case

Mehedinţi- 1 case

B. List of regions / countries where human cases with West Nile virus infection have been communicated so far:

1. Greece-regions **Kilkis, Thessaloniki**, Imathia, **Serres, Pieria, Pella, Larissa**
2. Italy - Emilia Romagna Region, **Veneto, Lombardy**
3. Israel
4. **Hungary**
5. Russia- **Volvograd**
6. **U.S.A**, Canada, Mexico

Annex 3. Photos from the mission

Picture 1: Debriefing by ECDC/WHO Regional Office for Europe mission team at Romanian Public Health Institute

Picture 2: Dr Florin Popovici (Romanian National Public Health Institute, Romania) and Dr Romeo Bellini (Centro Agricoltura Ambiente 'G. Nicoli', Emilia-Romagna Region, Italy)

Picture 3: Dr Roxana Serban (Romanian National Institute of Public Health) and Dr Cornelia Ceianu (National Institute for Research and Development in Microbiology and Immunology 'Cantacuzino')

Picture 4: Romeo Bellini (Centro Agricoltura Ambiente 'G. Nicoli', Emilia-Romagna Region, Italy), Ana Paula Coutinho (WHO Regional Office for Europe), Anca Sirbu (Romanian National Public Health Institute), Annick Lenglet (European Centre for Disease Prevention and Control)

Picture 5: WNV response team for Constanta County with Romeo Bellini (Centro Agricoltura Ambiente 'G. Nicoli', Emilia-Romagna Region, Italy) and Henriette de Valk (INVS, Paris, France)

Picture 6: Romeo Bellini (Centro Agricoltura Ambiente 'G. Nicoli', Emilia-Romagna Region, Italy), Leonid Carpus (Constanta District), and Roxana Serban (Romanian National Institute of Public Health)

Picture 7: Map used in Constanta county showing identified WNV mosquito vectors