The ecological surveillance of West Nile virus in Catalonia: in continuous evolution

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Abstract
An ecologic surveillance system for West Nile was implemented in Catalonia in 2007. This system consisted of different components: active and passive avian surveillance, follow-up of chicken sentinels, cross-sectional surveys in feral equines, follow-up of equine sentinels, passive equine surveillance, and entomological surveillance. Until 2010 these activities have been continuously adapted to improve the efficiency and the sensitivity of the surveillance. Between 2007 and 2010 the active WNV infection was not detected in any component.

Keywords: West Nile, surveillance, North-Eastern Spain.

Introduction
West Nile Virus (WNV) is a widespread zoonotic pathogen that maintains its transmission cycle principally between mosquitoes and birds. The circulation of this flavivirus has been frequently evidenced around Europe and the Mediterranean Basin. Recent outbreaks, such as epidemics in Greece or Rumania in 2010, have caused serious neurological disease in humans and equines and even fatal cases [1, 2].

In the south of Spain, its activity has been also recently detected in humans and horses and previous circulation of WNV had been also confirmed in diverse avian species and horses in this area [3, 4, 5].

To prevent the potential impact of epidemics in public and animal health there is a current need of implementing surveillance systems in areas at high risk in Europe and neighbouring countries [6].

In Catalonia (in north-east of Spain), which is close to neighbouring areas where WNV has been previously evidenced [1], exist important wetlands that serve as resting sites for many birds migrating between regions of Africa and Europe and which are exposed to a high density of culicids. In this region has been implemented an ecologic surveillance system for WNV since 2007.

The work describes the different strategies carried out between 2007 and 2010, the results obtained from each component, and discusses the strengths and weaknesses detected.

Materials and methods
The ecologic surveillance for West Nile implemented in Catalonia consists of avian, equine and entomological surveillance. These components were mainly implemented during the period of activity of adult culicids in the main wetlands considered at risk.

The active avian surveillance consisted in serological surveys. All the sera were tested by a competitive ELISA (cELISA), and confirmed by virus-seroneutralization test (VNT). Between 2007 and 2009 these samples were obtained from the bird ringing schemes that were run in chicks of Phoenicopterus roseus, Larus audouinii, and Larus michahellis in areas considered at risk. In 2010 the strategy of sampling was modified, and sera samples of a broad variety of different species were obtained from the wildlife rehabilitation centers who sampled birds from the whole region during all the period of study.

In 2008 the serological sampling of sentinel chickens was initiated as a new component. These samples were collected from backyard holdings located close to areas at risk. In each holding 5 chickens were bimonthly sampled between May and November.

The passive avian surveillance included investigations of birds found dead from peaks of mortality detected over the entire territory. Tissue of encephalon was collected from carcasses and subsequently tested through a specific real time RT-PCR (RRT-PCR) for WNV.

The equine surveillance was based on periodical serosurveys in sentinel, cross-sectional surveys in feral horses bred outdoors in the wetland areas, and passive surveillance. In relation to sentinel equines, a total of 17 holdings were followed up. In each holding sera samples were bimonthly collected from 4 seronegative unvaccinated horses, subsequently were screened by cELISA and confirmed by VNT.

The passive surveillance in equines was based on the investigation of those suspicions that showed neurological clinical signs. The sample of election was cerebrospinal fluid (CSF) or nervous tissue (mainly medulla oblongata). These samples were taken in collaboration with veterinarians and the services of carcass disposal that collected the nervous tissue at the moment of picking up and removing deceased horses. The sera and CSF samples were tested by cELISA, and CSF and nervous tissue by RRT-PCR.
The entomological surveillance was carried out in areas considered at high risk. Between 2007 and 2009 a homogeneous scheme of trapping was used in the different wetlands. This system allowed comparing the results among these areas. CDC light traps baited with CO₂ traps were set out fortnightly. These traps were located in three different sites based on the type of habitat: natural areas with vegetation of coastal wetlands with high density of wild birds, rural areas with crops (rice fields and others) and with high density of domestic animals, and urban or periurban areas. The specimens captured were identified and pooled into groups up to 40 mosquitoes, according to the date, site of collection and species. All these pools were processed to detect viral genome for the Flavivirus genus. This detection consisted in viral RNA extraction according to manufacturer (Qiagen) instructions, followed by a generic RT-nPCR. Subsequently, the positive pools for flavivirus were specifically tested for WNV by RT-PCR.

In 2010 the scheme of the entomological surveillance changed. A continuous monitoring using different types of traps was carried out. The goal was to determine the species and the dynamics of culicidae population in wetland areas. The viral detection in vectors was uniquely planned in the event of previous detection of WNV in vertebrates.

Results

The follow-up of the different components of surveillance implemented in Catalonia along this 4-year period is shown in Table 1 and Figure 2.

Table 1: Summary of the ecologic surveillance for West Nile implemented in Catalonia (2007-2010)

<table>
<thead>
<tr>
<th>Year</th>
<th>Wild birds</th>
<th>Chicken sentinels</th>
<th>Equines</th>
<th>Mosquitoes captured</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active surveillance</td>
<td>Passive surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>130</td>
<td>18</td>
<td>54</td>
<td>349</td>
</tr>
<tr>
<td>2008</td>
<td>95</td>
<td>21</td>
<td>100</td>
<td>43</td>
</tr>
<tr>
<td>2009</td>
<td>47</td>
<td>98</td>
<td>110</td>
<td>46</td>
</tr>
<tr>
<td>2010</td>
<td>78</td>
<td>15</td>
<td>125</td>
<td>87</td>
</tr>
<tr>
<td>Total</td>
<td>341</td>
<td>99</td>
<td>364</td>
<td>22083</td>
</tr>
</tbody>
</table>

Avian surveillance in wild birds

Between 2007 and 2010, 828 wild birds belonging to 71 species of 18 orders were tested for WNV. In total 537 sera were collected by active surveillance to detect the presence of antibodies, and 291 were collected by passive surveillance and tested by RT-PCR for WNV.

Between 2007 and 2009 none of the samples tested gave a positive result. In 2010, although none of the samples resulted positive by passive surveillance, 5 sera of species belonging to order Accipitriformes resulted positive by cELISA and VNT. Three of these positive birds were Short-toed Eagles (Circus galicatus) (1/20, 1/40, 1/60), one was a Black kite (Milvus migrans) (1/10) and one was a Red kite (Milvus milvus) (1/15). All of them are migrant species in the period of nesting in Catalonia that come from African countries. None of these positive birds was sampled in areas considered at high risk in Catalonia.

Equine surveillance

In total 572 equine sera coming from 17 sentinel holdings were tested between 2007 and 2010. Sixty-seven horses in 2007, 61 in 2008, 66 in 2009 and 67 in 2010 served as serological sentinels for WNV. All serological tests resulted negative except one indigenous that was positive to cELISA and VNT (1/10), and negative to the ELISA for Ig M. None of the feral horses included in cross-sectional serosurveys gave positive results.

Clinical suspicions in equines

Between 2007 and 2010, thirteen equines with clinical signs of encephalitis were reported and tested. All of them were negative to WNV by real time RT-PCR.

Entomological surveillance

A total of 22,083 mosquitoes were captured during this 4-year period. Between 2007 and 2009, 14,404 mosquitoes belonging to 15 different species were pooled in 902 groups. Sixty one pools resulted positive to Flavivirus genus, although none of them gave positive to WNV.

The most abundant species captured were: Culex pipiens (40.1%), Ochlerotatus caspius (37.5%), Culex modestus (12.3%), and Anopheles atroparvus (4%).

Discussion

From 2007 to 2009 none of the samples obtained from the components of ecological surveillance for WNV in Catalonia resulted positive. These results are in contrast with previous serosurveys performed in humans in the area of influence of Ebro Delta [7]. However it should be noticed that during this period the active surveillance in wild birds was mainly focused, both space and time. Thus, the results can not be inferred to the global population of wild birds in Catalonia. In contrast in 2010 the serological surveys conducted in wild birds from May to November in different species have shown that the WNV incursion is highly probable. This kind of sampling has demonstrated to be efficient to detect avian reservoirs and points out the need of amplifying the surveillance to other areas not considered at risk.
In relation with the negative results from the passive surveillance in wild birds, no peak of mortality caused by WNV infection was detected. However, unlike the WNV epidemic in the United States, observing the behavior of previous outbreaks occurred in other European areas, peaks of mortality in wild birds have not been a good indicator of the early circulation. Thus, the fact that WNV have not been detected in dead birds in Catalonia is not a sufficient indicator to discard viral circulation.

Regarding the use of chicken sentinels, this method has proved to be useful to the early detection. This is the case of northern Italy in 2005 where 90% of sentinel birds (56/62) seroconverted without being detected in any other population [10]. From 2008 to 2010 a total of 350 birds were used as sentinels, and none has been detected as positive. These results are in agreement with other components and indicate that in recent years the WNV circulation in domestic birds has been null or insignificant.

The detection of WNV in horses indicates a high rate of viral amplification in an area and poses a potential danger to public and animal health [8]. In Catalonia the unique indication of WNV circulation in equines was detected through serology in 2010. One of the equine sentinels resulted positive to IgG (1/10) and negative to IgM, indicating an old infection or cross-reaction. This result corroborates the importance of maintaining a continuous equine surveillance in the region to detect active circulation.

Regarding entomological surveillance, previous experiences showed that this is not a very sensitive component to detect the presence of WNV in the initial stages [10]. Nevertheless, this component provides itself valuable information to both identify the possible risk of spread in an area and in the event of outbreak to plan control measures. The results of entomological monitoring in these recent years show that species such as Culex pipiens, or Culex modestus, Ochlerotatus caspius, which are known as competent vectors for WNV, are widely distributed in Catalonia. Moreover, the introduction and wide spread of Aedes albopictus in this region constitutes an important concern due to its vectorial capacity and its aggressive behaviour in human biting. In relation with the WNV genome monitoring in vectors it should be pointed out that the circulation WNV has not been detected; however other flaviviruses with an unknown importance in public or animal health were frequently detected mainly in Aedes caspius and Culex pipiens in areas with a high density of wild birds.

From our results we believe that components as theserological avian surveillance from the wild rehabilitation centers, the active and passive equine surveillance, and the continuous entomological monitoring could serve as the best indicators of the risk of WNV spread in our region. Anyway, on the basis of our experience, the surveillance for WN should be maintained as a flexible system under constant evaluation. And its design requires a continuous adaption according to the epidemiological situation, the disponibility of resources and the technical capacities of the network of professionals involved in each task.

References

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