WEST NILE VIRUS SURVEILLANCE IN CATALONIA 2007 *

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RESUME

La surveillance du virus de la fièvre du Nil occidental a été mise en place en Catalogne (Nord-Est de l'Espagne) en 2005, ayant comme principal objectif la détection précoce de la circulation du virus du Nil occidental (VNO) chez les vertébrés hôtes et chez les vecteurs. Ce programme a été mis en œuvre dans les trois principales zones humides de la région, au cours de la période de grande activité des *Culicidae.*

Il a été fondé sur les vertébrés hôtes de surveillance, oiseaux sauvages et équidés, et sur un système de surveillance entomologique.

La surveillance active et passive a été mise en œuvre chez les vertébrés hôtes. La surveillance passive est fondée sur le dépistage des oiseaux morts et sur les chevaux avec des signes cliniques compatibles. La surveillance active a été mise en œuvre chez les oiseaux sauvages à risque accru et chez des chevaux sentinelles. En 2007, un total de 98 échantillons provenant d'équidés et 236 échantillons d'oiseaux sauvages ont été collectés et analysés.

Un système de surveillance entomologique a été également mis en œuvre. Ce système permet d'obtenir des informations sur la population de *Culicidae*, et de détecter l'ARN des arbovirus. En 2007, un total de 3 846 moustiques ont été testés.

Au cours de l'année 2007, la circulation du VNO en Catalogne n'a pas été détectée dans aucun des composants du reseau de surveillance.

Mots-clés : Epidémiosurveillance, virus du Nil occidental, oiseaux sauvages, chevaux, *Culicidae,* Catalogne.

SUMMARY

The West Nile Virus surveillance programme was implemented in Catalonia (North-Eastern Spain) in 2005, having as a main goal the detection of the early circulation of West Nile Virus (WNV) in vertebrate hosts and vectors. This programme was implemented in the three main wetlands of the region during the period of major activity of *Culicidae* mosquitoes.

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The programme was based on vertebrate host's surveillance, as wild avian species and equines, and an entomological surveillance system

Both passive and active surveillance were implemented in vertebrate hosts. The passive surveillance system was based on the analysis of dead birds or suspected horses with compatible clinical signs, and the active surveillance system in wild birds at increased risk and equine sentinels was based on a serological response. In 2007, a total of 98 samples from equines and 236 samples from wild birds were collected and tested.

An entomological surveillance system was also implemented allowing to get information about the *Culicidae* population indexes, and to detect arboviral RNA genome for the genus *Flavivirus.* In 2007*,* a total of 3,846 mosquitoes were tested.

During the year 2007 the circulation of WNV in Catalonia was not detected in any component of the surveillance system.

Keywords : Surveillance, West Nile Virus, Wild birds, Equines, *Culicidae*, Catalonia.

I - INTRODUCTION

In the recent years, arthropod borne diseases have increased their importance in the Western Hemisphere. The implementation and evaluation of different surveillance systems of many emerging arboviruses, as West Nile Virus (WNV) around Europe, has become a necessity due to the potential impact of these viruses in public and animal health.

In Catalonia, a region located in the North-Eastern Spain, a WNV surveillance programme has been implemented since 2005. Some factors justify the programme: first of all, there are areas at high risk that can easily maintain the vector-host transmission cycle, and that have a high density human population, on the other hand there are past records of the WNV circulation in neighbouring areas of Portugal, Southern Spain, France. In Spain, WNV specific antibodies have been detected in humans in Catalonia (Bofill et al., 2006), and in birds in the South-Western Spain [Figuerola *et al*., 2007] ; in France two outbreaks were described in 2000 affecting horses [Murgue *et al*., 2001], and in 2003 affecting humans and equines [CNR des Arbovirus et Afssa, 2003], also serological response was observed in horses in 2000 [Durand *et al*., 2002]. In Portugal, WNV genomic RNA was detected in mosquito pools [Esteves *et al.*, 2005].

II - DESIGN OF THE WNV SURVEILLANCE SYSTEM IN CATALONIA

1. OBJECTIVE

This surveillance programme was mainly focused on the early detection of WNV circulation in wild birds and equines and *Culicidae* mosquitoes from May to November in the areas considered at higher risk.

2. HIGH RISK AREAS

The areas were selected taking into account the wild bird census, their location in migratory flyways and a high density of *Culicidae* population. These areas were: *Aiguamolls de l'Empordà*, *Delta del Llobregat* and *Delta de l'Ebre* (see figure 1).

Figure 1

Areas where the WNV surveillance system was implemented

3. TARGET POPULATION

The target populations of the WNV surveillance system were: equines that were bred into the areas at high risk, which included in total 176 holdings with 2,996 horses, the wild avian species considered at risk by the Avian Influenza surveillance system (see table 1), and the *Culicidae* mosquito population.

Table 1

Census of wild birds [Source: DAMiH, 2006]

	WETLANDS AREAS		
FAMILIES	**Aiguamolls de l'Empordà**	**Delta del Llobregat**	**Delta de l'Ebre**
Anatidae + Rallidae	16,123	7,015	113,033
Laridae + Sternidae	31,591	30,627	25,606
Other families	12,202	9,351	96,060
Total Census	**59,916**	**47,053**	**234,699**

4. EQUINE SURVEILLANCE

Two components were considered for the WNV surveillance in equines: sentinels and passive surveillance.

Horses are good sentinels for WNV infection surveillance as they are easily identifiable, act as dead-end hosts and there are available serological tools [Anonymous, 2003].

The sentinels were located in the three risk areas. Bimonthly, four individual animals in each of the 16 sentinel holdings were bleeded. The laboratorial test used for the detection of WNV antibodies was a commercial competitive ELISA (IdVet ®) against IgM and IgG, whereas the confirmation technique was the plaque reduction neutralisation test (PRNT) [OIE, 2004].

The passive surveillance was based on the investigation of the clinical suspects and the samples of election were cerebral spinal fluid or nervous tissue (mainly medulla oblongata).

On those samples, a competitive ELISA and a real time RT-PCR [Jiménez-Clavero *et al*., 2006] were performed.

5. AVIAN SURVEILLANCE

Two approaches were used: a passive surveillance system based on the analysis of dead birds, and an active surveillance system performed during the campaigns of bird ringing in wild birds at increased risk based. The type of collected sample for the passive surveillance was tissue of the encephalon and these samples were tested through a specific real time RT-PCR for WNV [Jiménez-Clavero *et al*., 2006], whereas for the active surveillance sera were collected and tested by competitive ELISA (IdVet ®) and the plaque reduction neutralisation test (PRNT) [OIE, 2004].

6. ENTOMOLOGICAL SURVEILLANCE ON *CULICIDAE* GENUS

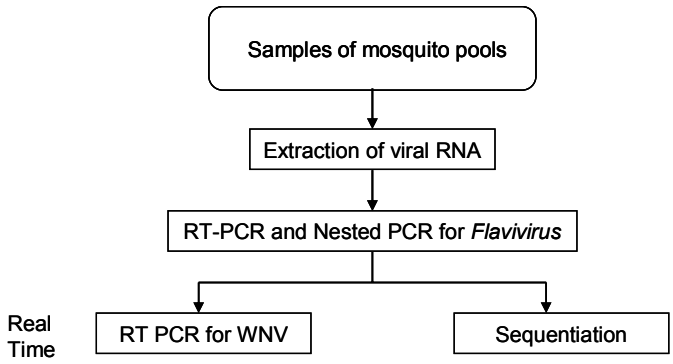
The mosquito surveillance system allowed us to get information about the *Culicidae* population indexes and to detect arboviral RNA molecules directly from mosquito homogenates. The protocol consisted of a RNA extraction (Qiagen) following manufacturer instructions, followed by a RT-nPCR for the genus *Flavivirus* [Sánchez-Seco *et al*., 2005]. Those pools which were positive for genus *Flavivirus* were tested later for *WNV* specifically by real time RT-PCR [Jiménez-Clavero *et al*., 2006] (see in figure 2 the diagnostic protocol scheme to detect RNA genome of *Flavivirus* genus and WNV).

The mosquito specimens were captured using CDC light traps baited with CO_2. The traps were located in three types of sites in each wetland: 1- areas with high density of wild birds, 2- areas with a high density of equines and poultry and 3- zones close to urban areas. The trapping frequency was every two weeks from May to October.

The specimens were identified and pooled according to date of collection, location and specie.

Figure 2

Laboratorial diagnosis to detect RNA genome of genus *Flavivirus* and WNV, specifically



II - RESULTS

During the year 2007, a total of 98 equine samples were collected and only two of them were obtained through passive surveillance. A total of 236 samples belonging to different species of wild birds samples were collected, 130 samples obtained through active surveillance and 106 through passive surveillance. In relation to the entomological surveillance, 3,846 mosquitoes were captured and grouped in 294 mosquito pools.

Figure 3 shows the period along 2007 in which the different samples of the surveillance system were collected.

Figure 4 shows the geographical distribution of the sampling for each component in the main three wetlands and the total samples collected in each one of them during 2007.

Figure 3

Collected samples along 2007 in the WNV surveillance programme in Catalonia

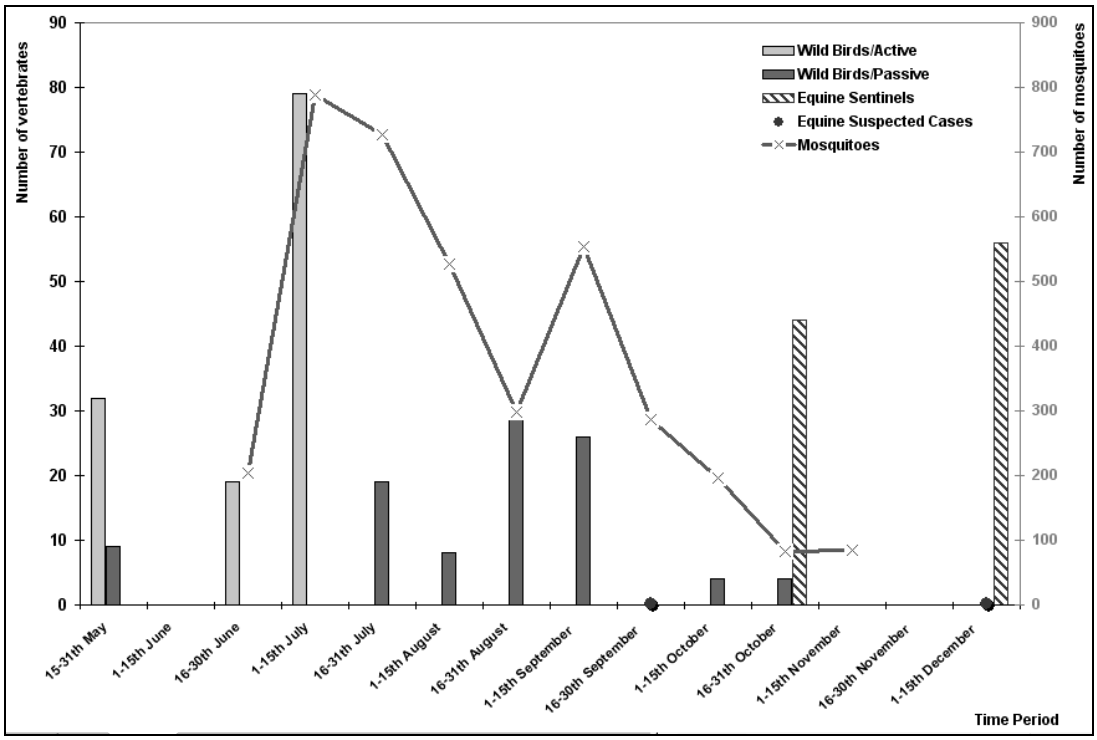
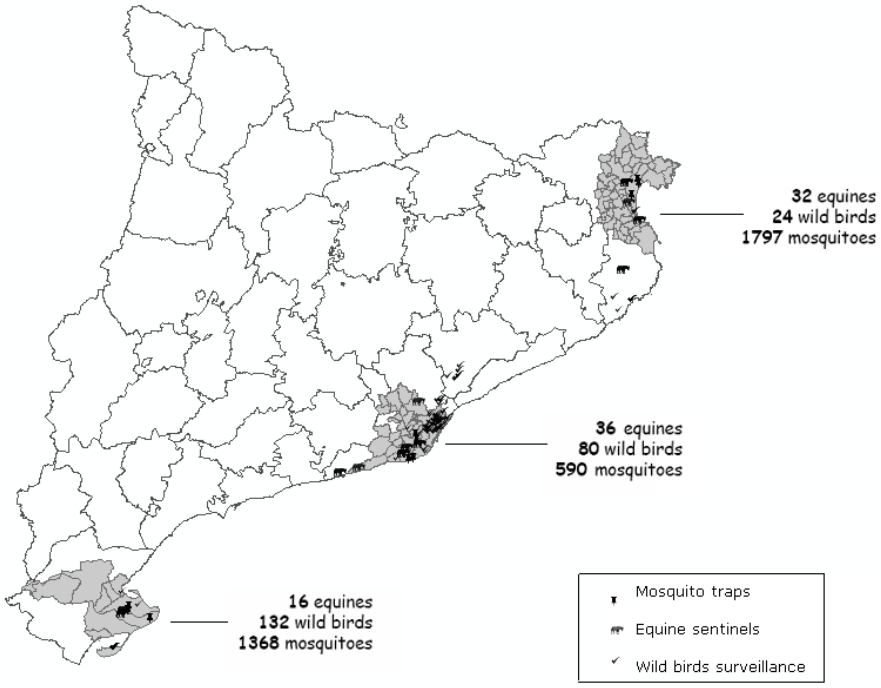


Figure 4

Spatial distribution of the sampling for each component in the main three wetlands during 2007



The collected samples of wild birds belonged to different species. Table 2 shows the species that were sampled along 2007 differentiating the type of surveillance used to collect them.

All the results of the submitted equine and avian samples were negative.

In relation to the entomological surveillance, the most representative species were *Culex pipiens, Aedes caspius* and *Anopheles atroparvus*. From 294 collected pools, 20 were positive for genus *Flavivirus*, although none of them were WNV positive. Actually, the importance of these detected *Flaviviruses* linked to public and animal health remains unknown. The table 3 shows the captured mosquito species along 2007 and the obtained results.

Table 2

Type and number of avian species tested during 2007

Sampled species	Active surveillance	Passive surveillance	Total
Alcedo atthis		1	1
Anas platyrhynchos		77	77
Anas sp		3	3
Anas domesticus		3	3
Anser anser		3	3
Cygnus Olor		1	1
Egretta garzetta		1	1
Gallinula chloropus		2	2
Himantopus himantopus		2	2
Ixobrychus minutus		1	1
Larus audouinii	19		19
Larus cachinnans		1	1
Larus michahellis	32		32
Larus ridibundus		3	3
Phoenicopterus ruber	79		79
Puffinus yelkouan		5	5
Streptopelia turtur		1	1
Tachybaptus ruficollis		1	1
Tringa totanus		1	1
Total samples	**130**	**106**	**236**

During 2006 the WNV entomological surveillance system permitted to detect the circulation of the Usutu virus among *Culex pipiens* in Delta del Llobregat [Busquets *et al*., 2007], although none unusual peak of mortality in wild birds was detected; whereas during 2007 Usutu virus occurrence in mosquitoes was not detected.

The highest quantities of *Culicidae* mosquitoes were captured in Aiguamolls de l'Empordà followed by Delta de l'Ebre. In Aiguamolls de l'Empordà it was also obtained the highest amount of positive results, mainly in *Aedes caspius* and *Culex pipiens* specie.

Most of the positive results were obtained from the traps located in the areas whit high wild birds density. Figure 5 shows the obtained results through the entomological surveillance differentiating each wetland and the different ecological sites in each one of them.

Table 3

Mosquito species collected and tested along 2007. Twenty pools were positive for genus *Flavivirus*, but no sample was positive for WNV.

Sampled species	Number of mosquitoes	Number of pools	Number of positive pools to genus *Flavivirus*
Aedes albopictus	98	30	0
Aedes vexans	1	1	0
Alopecurus geniculatus	1	1	0
Anopheles atroparvus	273	23	0
Anopheles hyrcanus	1	1	0
Coquillettidia richiardii	4	3	0
Culex modestus	247	20	0
Culex pipiens	1 988	123	**5**
Culex theileri	47	5	0
Culiseta anulata	5	1	0
Culiseta longiareolata	2	2	0
Culiseta subochrea	5	3	0
Aedes caspius	1 166	77	**14**
Ochlerotatus geniculatus	1	1	0
Ochlerotatus pulcritarsis	6	2	0
Uranotaenia unguiculata	1	1	**1**
Total	**3 846**	**294**	**20**

III - DISCUSSION

The WNV surveillance involves a wide range of hosts, including domestic and wild species. This fact makes difficult to inference the results to the reality of the target population.

In the equine surveillance system only two cases were passively reported. Some future campaigns to inform the horses' owners and veterinarians can help to improve the sensitivity of this component.

The difficulty in the avian surveillance system to get samples from the wild birds made that the choice of the samples was often by convenience or haphazard methods and as a consequence the representativeness of the sampling decreased.

Comparison among the obtained results of the entomological surveillance between each wetland and the ecological sites in each one, arose some differences. These results can be useful to estimate roughly the risk of the different areas or sites. Furher investigation would be necessary in order to evaluate the factors that may have an influence on these detected differences.

The WNV surveillance system allowed establishing a multidisciplinary professional network to monitor the circulation of possible emerging arboviruses in Catalonia and at the same time to characterise some population at risk and the possible vectors that can be involved in the spreading of these viruses.

Figure 5

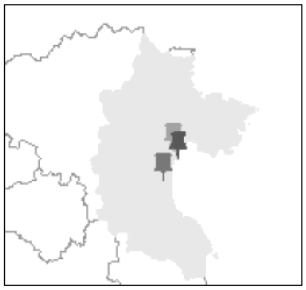
Results of the entomological surveillance differentiating each wetland and the different ecological sites in each one of them

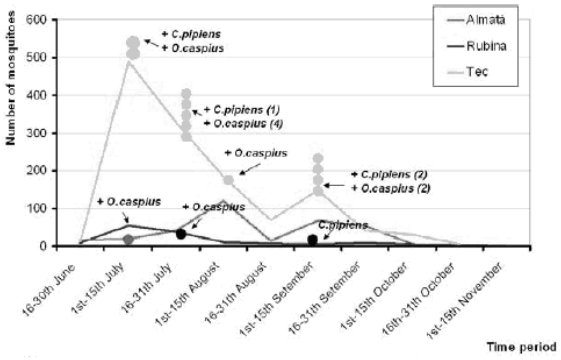
Type of sites

- with high density of wild birds
- with horses and poultry holdings
- close to urban areas

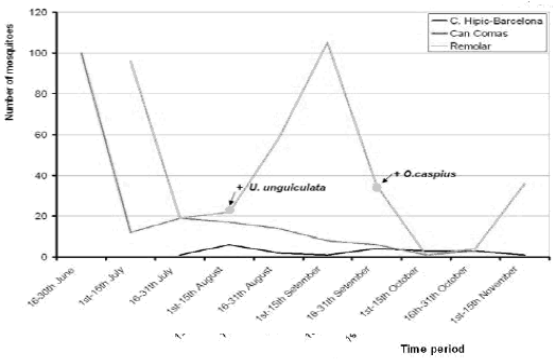
Wetland	Number of mosquitoes	Number of pools	Number of positive pools
Aiguamolls de l'Empordà	1747	103	14
Delta del Llobregat	590	48	2
Delta de l'Ebre	1368	111	4

Aiguamolls de l'Empordà

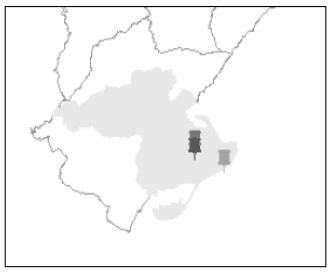


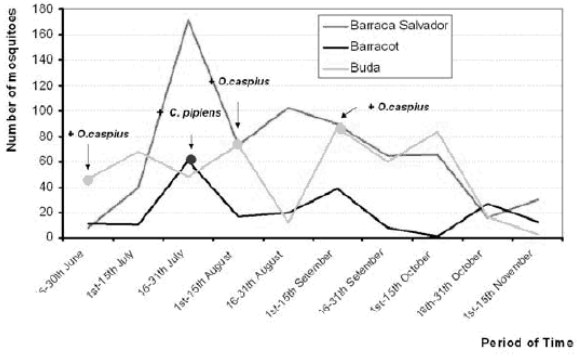


Llobregat Delta



Ebre Delta





IV - CONCLUSION

The WNV circulation in equines, wild birds and mosquitoes was not detected in Catalonia in 2007.

Other Flaviviruses with an unknown importance in public or animal health were circulating mainly in *Aedes caspius* and *Culex pipiens* in areas with a high density of wild birds. This circulation was significantly higher in Aiguamolls de l'Empordà.

In 2006, the circulation of Usutu virus was detected in *Culex pipiens* in Delta del Llobregat, although none abnormal peak of mortality in avian was appreciated. During 2007, Usutu virus occurrence was not detected.

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