Evaluation of West Nile virus surveillance and early warning system in Greece, based on domestic birds

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Because of their role in spreading WNV, **birds are useful candidates as sentinels** for the presence of the virus in a geographical location.
Mosquito population

Infected mosquitoes

Overwintering mosquitoes

Viraemic birds

larviciding

adulticiding

Human cases

Spring

Summer

Autumn

Winter

WNV epidemiology
Four categories of birds can be used:
1. dead wild birds,
2. trapped wild birds,
3. captive sentinel birds,
4. domestic sentinel birds
Appropriate surveillance schemes in the context of lineage 2 WNV circulation in Greece?

<table>
<thead>
<tr>
<th>Surveillance method</th>
<th>Disease Activity</th>
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<tbody>
<tr>
<td>Human cases</td>
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<td>Veterinary cases (horses)</td>
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<td>Mosquitoes,</td>
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<td>sentinel birds</td>
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<td>Dead bird surveillance</td>
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In Greece abnormal bird mortalities were not observed.
Aim
Provide data on **lineage 2 WNV enzootic circulation**, in Central Macedonia, Greece

**Surveillance systems**
1. domestic pigeons,
2. free-range chicken,
3. captive chicken
Questions

a) Can we assess the geographical spread of WNV, immediately after the epidemic seasons, using WNV antibodies in juvenile domestic birds as indicators of WNV circulation?

b) Is there a correlation between the WNV point seroprevalence in birds and the incidence rates of human WNND cases?

c) What is the early warning capacity of domestic bird surveillance systems
Domestic pigeons and free-range chickens

Determination of WNV point seroprevalence in juvenile birds and correlation with incidence rates of human WNND after the epidemic seasons

End of 2010 and 2011 epidemics:
pigeons younger than 12 months old were tested

End of 2011 and 2012 epidemics:
juvenile free-range chickens were tested

- C-ELISA (IDVet ID Screen West Nile Competition)
- micro-PRNT (neutralizing IgM, IgG)
Geographical spread of WNV based on point seroprevalence in juvenile pigeons after the end of 2010 epidemic (Oct. 2010 – Feb. 2011), n=655 pigeons (131 pigeon pens, 5 birds/pen)
Central Macedonia after the end of the epidemics
The incidence of reported human WNND cases by prefecture of residence was correlated to the pigeon juvenile seroprevalence (per 100,000)

**A**
Regional unit
2010

**B**
Municipality
2010

**C**
Regional unit
2011

**D**
Municipality
2011

\[ \rho = 0.94, P\text{-value} < 0.001 \]

\[ \rho = 0.54, P\text{-value} = 0.01 \]

\[ \rho = 0.56, P\text{-value} = 0.32 \]

\[ \rho = 0.32, P\text{-value} = 0.47 \]
What is the early warning capacity of domestic bird surveillance systems?
Evaluation of the early warning capacity of domestic birds and detection of the circulating WNV strain

- Pigeons
- Free range chickens

Sampling was performed before human case onset

**bird age: 1.5-5 months** (avoidance of maternal antibodies and exposure to the previous epidemic)

Exact **sample collection time** from pigeons **varied** in order to increase the probability of detection of WNV enzootic transmission early in the season

- based on **local temperatures** and **mosquito population data**
All collected sera were assayed by cELISA

- cELISA-positive sera were confirmed by micro-VNT against WNV
- positive sera were examined by real-time RT-PCR
Pigeons, early warning 2011 (sampling, June 15 - July 29)
n= 270, (54 pens, 5 birds/pen)

- 20 seropositive birds (15/6-6/7) identified prior to human cases
- First seroconverted birds detected 1.5 months prior to human cases
Free range chickens, early warning 2011 (June 16 - July 5) 
n= 120, (24 chicken coops, 5 birds/coop)

- 26 seropositive birds (7/6-22/7) before human case onset
- First seroconverted birds were detected 1.5 months prior to human cases
Pigeons, early warning 2012
(sampling June 6-July 9)
n= 240, (48 pens, 5 birds/pen)

- 12 seropositive birds (13/6-22/6) before human case onset
- First seroconverted birds were detected 1.5 months prior to human cases
Free range chickens, early warning 2013 (June 14 - 28)  
n= 205, (41 chicken coops, 5 birds/coop)  

- 8 seropositive birds (14/6-28/6) before human case onset  
- First seroconverted birds were detected 1.5 months prior to human cases
Early warning capacity of domestic birds

<table>
<thead>
<tr>
<th>Pigeons</th>
<th>% seroconverted pigeons</th>
<th>Incidence in humans per 100.000</th>
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<tbody>
<tr>
<td>2011</td>
<td>7%</td>
<td>1,0</td>
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<tr>
<td>2012</td>
<td>5%</td>
<td>0,8</td>
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<th>Chicken</th>
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<tr>
<td>2011</td>
<td>22%</td>
<td>0,8</td>
</tr>
<tr>
<td>2013</td>
<td>4%</td>
<td>0,3</td>
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**Molecular detection** and characterization of the circulating WNV strain **was not possible** in any of the sera tested. This was anticipated, since the duration of viremia in pigeons is short (3-4 days) and in our surveys, pigeons were sampled only once.
Domestic pigeons and free-range chickens

Advantages

- **Convenient** and **inexpensive** (no costs for setting up and maintaining the flocks)
- The geographic location of infection is not in question
- The system is **flexible** (it can be expanded and contracted as appropriate)
- The high number of areas surveyed enabled us to determine foci of WNV early enzootic circulation in areas of Central Macedonia, west of Axios River
- Early warning was possible for 3 consecutive years (timely dissemination of information to public health authorities, to increase preparedness and implementation of vector control measures)

Disadvantages

- Molecular characterization of the circulating virus is very difficult to achieve
- Weekly sampling and monitoring of WNV circulation is difficult
Weekly sampling (serum, plasma) from May to October

Determination of seroconverted birds (ELISA/PRNT)

RT-PCR analysis retrospectively in samples collected from seroconverted birds
Repetitive sampling in captive chickens (2011)

7 cages x 6 chickens at the edges of Thessaloniki City
Evidence of enzootic circulation of West Nile virus (Nea Santa-Greece-2010, lineage 2), Greece, May to July 2011

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2. Aristotle University of Thessaloniki, Faculty of Veterinary Medicine, Laboratory of Microbiology and Infectious Diseases, Thessaloniki, Greece
3. American Farm School, Thessaloniki, Greece

11 chickens seroconverted (10 in Western Thessaloniki)

WNV detected by real-time RT-PCR in 2/11 birds

- NS3 sequencing
- Virus isolation

Nea Santa-Greece-2010 lineage 2 WNV (99.9% identity)
WNV enzootic circulation was detected one month prior to the onset of human cases.
Repetitive sampling in captive chickens (2012)

Human WNND cases
Chicken cages
Seropositive chickens
West Nile Virus Lineage 2 Strain in Greece, 2012

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ML phylogenetic analysis based on complete NS3 nt sequences (1863bp) of lineage 2 WNV strains

Only a few synonymous nucleotide substitutions consisting of transitions were identified, indicating minimum evolution of the virus during the 2010-2012 period
Captive Chickens

Advantages

- Weekly monitoring of WNV activity in transmission foci
- Fast isolation and molecular characterization of the circulating virus, determination of strain virulence and estimation of public health risk (important in areas with different WNV strains)
- Early warning is possible

Disadvantages

- Determination of enzootic transmission foci at which cages should be placed is a prerequisite
- Only focal transmission is detected (multiple flocks should be positioned in representative geographic areas)
- Increased cost of setting up and maintaining flocks
- Flocks are subject to vandalism and theft
3-year experience from Central Macedonia area
Mosquito control in Central Macedonia

- Sentinel bird–based WNV surveillance systems have provided evidence of Initial enzootic transmission foci of WNV 1.5 months before the occurrence of human cases.
- Some of the detected domestic bird seroconversions might have occurred long before the onset of the samplings.
- After the infection, more than 7 days are needed for the detection of seroconversion by ELISA.
- A mosquito requires approximately 10-14 days after its initial blood meal to become infectious and to transmit WNV to other animals (extrinsic incubation period).

Amplification of the primary enzootic cycle of WNV occurred at least 2 months before the occurrence of human cases (May?).

Early application of larviciding!!!!
Απαιτείται έγκαιρη και αποτελεσματική μείωση των πληθυσμών των κουνουπιών στα πλαίσια ολοκληρωμένων προγραμμάτων διαχείρισης

Στόχος:
Η μείωση της έντασης της επιζωτίας στα πτηνά.
   Αναμένεται να μειώσει και τον αριθμό των μολυσμένων από τον ιό κουνουπιών και να συμβάλει σε αποτελεσματικότερη πρόληψη της νόσου στον άνθρωπο

Κεντρική Μακεδονία
Ανάγκη έγκαιρης και αποτελεσματικής καταπολέμησης των κουνουπιών τουλάχιστον δύο μήνες πριν την εμφάνιση των ανθρώπινων κρουσμάτων, δηλαδή Μάιο.

Στη συνέχεια η καταπολέμηση θα πρέπει να κατευθύνεται σε τοπικό επίπεδο με βάση τις μετρήσεις των πληθυσμών των κουνουπιών και την επιτήρηση σε πτηνά δείκτες.
Thank you for your attention!

ARISTOTLE UNIVERSITY OF THESSALONIKI

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