A diagnostic approach to wild bird surveillance and environmental sampling

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Diagnostic approach to wild bird surveillance and environmental sampling

- Many data are available concerning LPAI ecology in wild birds
- The HPAI ecology in wild birds is unknown
- The evolving AI situation requires flexible approaches

Avian Influenza in wild birds:
main objectives of the surveillance

1. To determine the circulation and the prevalence of AI viruses (including H5 and H7 LP/HP NAI)
2. To determine the presence or absence of HPNAI (e.g. A/H5N1) in the population of a certain area (early warning system)

Diagnostic approach in AI surveillance

STEP 1: samples collection

- Target population
  Species at risk for AI infection have been indicated in EU decision (2005/726/CE)

- Proper material
  cloacal swabs Vs tracheal swabs, internal organs

- Proper storage and shipment
  transport medium, liquid nitrogen; dry-ice
### Wild birds surveillance (active surveillance) & Targeted surveillance (passive surveillance)

<table>
<thead>
<tr>
<th>Area</th>
<th>Samples n</th>
<th>Sample type</th>
<th>LPAI (in civet)</th>
<th>HPAI (WB/DB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITALY</td>
<td>&gt; 4,000</td>
<td>&gt; Cloacal swabs</td>
<td>Many subtypes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>@ 8,000 (WB/DB)</td>
<td>Carcasses/live birds</td>
<td>Some subtypes</td>
<td>A/H5N1 (WB only)</td>
</tr>
<tr>
<td>EU (up to 12/2005)</td>
<td>&gt; 24,000</td>
<td>&gt; Cloacal swabs</td>
<td>Many subtypes</td>
<td>0</td>
</tr>
<tr>
<td>EUR</td>
<td>NA</td>
<td>Carcasses/live birds</td>
<td>NA</td>
<td>A/H5N1 (WB/DB)</td>
</tr>
<tr>
<td>AFRICA</td>
<td>5,000</td>
<td>&gt; Cloacal swabs</td>
<td>Various subtypes (H5 incl.)</td>
<td>0</td>
</tr>
<tr>
<td>AFRICA</td>
<td>@ 700</td>
<td>Carcasses/live birds</td>
<td>NA</td>
<td>A/H5N1 (DB)</td>
</tr>
</tbody>
</table>

WB wild birds / DB domestic birds. NA Not available.

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### INFLUENZA VIRUS SURVEILLANCE IN WILD BIRDS IN ITALY: RESULTS OF LABORATORY INVESTIGATIONS IN 2003-2005

<table>
<thead>
<tr>
<th>Species</th>
<th>N. Samples collected</th>
<th>N. positive in rRT-PCR</th>
<th>Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallard</td>
<td></td>
<td></td>
<td>H1N1, 2 x H4N6, 4 x H7N7 LPAI, H5N1 LPAI, H7N4 LPAI, H5N2 LPAI</td>
</tr>
<tr>
<td>Teal</td>
<td>442</td>
<td>94</td>
<td>2 x H7N7 LPAI, H5N3 LPAI, H5N2 LPAI</td>
</tr>
<tr>
<td>Pintail</td>
<td>136</td>
<td>6</td>
<td>H10N4</td>
</tr>
<tr>
<td>Snipe</td>
<td>46</td>
<td>2</td>
<td>H10N7</td>
</tr>
<tr>
<td>Other species</td>
<td>1678</td>
<td>148</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>430</td>
<td>15</td>
</tr>
</tbody>
</table>
| Percentage (N positives): | 11.7% | 3.5 (0.4) | 6th International Symposium on AI, Cambridge, UK, 2006

### H5N1 HPAI confirmed cases in wild birds (Italy, Feb 2006)

- Suspected cases (mortality in species at risk): 44
- Confirmed cases: 16 (wild swans and mallard)

### Step 2 - Diagnostic tools for AI surveillance

- Serology (AGID, HI, commercial kits)
- Virus isolation (embryonated eggs, cell cultures)
- Rapid Ag-immunoassays (commercial kits)
- Molecular methods (RT-PCR; Real-time PCR)
Serology in wild birds surveillance

- not recommended.
  No validated tests for the species involved (AGID, Immunoassay)
  Useful information provided (AGID, Immunoassay, HI)

  The detection of specific antibodies to H5N1 indicate a contact of the birds (reservoir) with any virus of the indicated subtypes.

  Due to the rapid and high mortality, serology appears of limited utility in HPAI infections.

Virus detection in wild birds surveillance

- Virus isolation
  Gold standard.
  It allows full characterization of the isolate.
  Viable virus is requested.
  Determination of viral infectivity is possible.
  Indicated for environmental samples.
  Difficult to manage for high throughput.

Virus detection in wild birds surveillance

- Molecular methods
  High sensitivity and specificity
  Rapid characterization of the isolate.
  Viable virus is not requested.
  Determination of viral infectivity is not possible.
  Adaptable to high throughput.
  Protocols and results interpretation are not harmonized

Virus detection in wild birds surveillance

- Ag-Immunoassay
  Sensitivity and specificity are reduced compared to previous methods (80-90%).
  Rapid indication on virus presence. Information limited to type A viruses.
  Viable virus is not requested.
  Not always adaptable to high throughput.
  No evaluation for their use in wild birds. No validation data are available.
  Careful selection of the material to be tested.
**Avian Influenza surveillance in wild birds:**
step 3 - developing a diagnostic approach

1. To determine the circulation and the prevalence of AI viruses (including H5 and H7 LPNAI)
2. To determine the presence or absence of HPNAI (e.g. A/H5N1) in the population of a certain area

### Diagnostic approach 1
To determine circulation and prevalence of AI viruses in wild birds

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Test Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotacal/OPh/Tracheal swab</td>
<td>Fecal dropping</td>
</tr>
<tr>
<td>Screening test*</td>
<td>Molecular methods (type A generic)</td>
</tr>
<tr>
<td>Virus isolation</td>
<td>Typing &amp; Subtyping confirmation</td>
</tr>
</tbody>
</table>

* Serology and Ag-immunoassays not recommended

### Diagnostic approach 2
To determine the presence of HPAI viruses in wild birds

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Test Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples (vegetative organs) from Respiratory &amp; Digestive tract of suspected cases</td>
<td>Rapid Ag-immunoassays* (preliminary indication)</td>
</tr>
<tr>
<td>Transport medium</td>
<td>Molecular methods (subtype specific)</td>
</tr>
</tbody>
</table>

* Ag-immunoassay results should be confirmed by PCR and/or VI

### Diagnostic approach to wild bird surveillance and environmental sampling - conclusions 1

1. The objectives of the surveillance should be established in advance, according to the priorities.
2. Diagnostic approach should be developed according to point 1.
3. Commonly available and easy-to-perform methodologies (e.g. serology and Ag-immunoassays) may generate inconclusive results.
Diagnostic approach to wild bird surveillance and environmental sampling - conclusions 2

• Objective 1 is of primary importance because of the need to improve our knowledge in LPAI/HPAI ecology in wild birds.
• Objective 2, as ”early warning system”, needs to be improved and based on data from objective 1.
• Diagnostic tests for surveillance should be harmonized (technical protocols & interpretation).

Diagnostic approach to wild bird surveillance and environmental sampling - conclusions 3

• Virus isolation can be delayed but never abandoned
  • Confirmatory test
    • Collection of isolates for
      » Pathogenesis studies
      » Challenge studies
      » Vaccine strain selection
      » Detailed antigenic and genetic information

• Sharing of information & data between countries and among scientific communities is of vital importance for the correct approach in AI surveillance and control.

Acknowledgement

• FAO/EMPRES, Rome, IT
• Staff members of the OIE/FAO Reference Laboratory, IZSVe, Legnaro, IT
• Dr. N.Gaidet & staff members of CIRAD, France
• Dr. V. Gubetti & staff members of INFs, IT
• The Italian Minister of Health