Epidemiology of wildlife diseases in Italy with particular reference to zoonotic agents

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Pavia, Italy
Relevance of the monitoring of wildlife diseases

According to OIE,

"the international community as a whole needs to monitor, prevent and control animal diseases in wildlife, a crucial component of the safeguarding of global animal and public health and related agriculture and trade issues."
Relevance of the monitoring of wildlife diseases

Global movements and exchange of pathogens within and between the populations of domestic and wild animals are in fact increasing.

Contact between the populations may occur in common grazing areas.
Relevance of the monitoring of wildlife diseases

Environmental conditions also largely influence the crossing of the species barrier by pathogenic agents.

Climate change also leads to changes in wildlife behaviour and migratory patterns.
Wildlife health is a cause for common concern for different stakeholders, such as:

- veterinary services
- public administration
- hunters
- gamekeepers
- farmers
- conservationists
- general public
Italy

- Area: 301,338 km$^2$
- Population: ~ 60 millions
- 20 regions
- 110 provinces
Lombardy and Emilia-Romagna
(two regions in Northern Italy)

- Area: 23,861 km²
- Population: 9,714,640 (09/2008)

- Area: 22,123 km²
- Population: 4,293,825 (12/2008)
IZSLER, Sezione di Pavia, Italy

Game Meat Hygiene in Focus, Brno, Czech Republic, 18-19 June 2009
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Governmental, bi-regional multi-provincial laboratory which provides

- analyses of animal samples
- analyses of foodstuffs
  - microbiological contamination
  - residues
- consultancy to stockbreeders, farmers, owners of animals
- technical assistance to other governmental institutions
- research
Some wild mammals of Lombardy and Emilia-Romagna (mentioned in this presentation…)

<table>
<thead>
<tr>
<th>Category</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruminants</td>
<td>roe deer (<em>Capreolus capreolus</em>), alpine chamois (<em>Rupicapra rupicapra</em>), red deer (<em>Cervus elaphus</em>), ibex (<em>Capra ibex</em>)</td>
</tr>
<tr>
<td>Canids</td>
<td>red fox (<em>Vulpes vulpes</em>)</td>
</tr>
<tr>
<td>Suids</td>
<td>wild boar (<em>Sus scrofa</em>)</td>
</tr>
<tr>
<td>Mustelids</td>
<td>badger (<em>Meles meles</em>), beech marten (<em>Martes foina</em>), pine marten (<em>Martes martes</em>), stoat (<em>Mustela erminea</em>), weasel (<em>Mustela nivalis</em>)</td>
</tr>
<tr>
<td>Lagomorphs</td>
<td>European hare (<em>Lepus europaeus</em>), mountain hare (<em>Lepus timidus</em>), wild rabbit (<em>Oryctolagus cuniculus</em>)</td>
</tr>
<tr>
<td>Rodents</td>
<td>mice (<em>Apodemus</em> spp., …), voles (<em>Microtus</em> spp., <em>Arvicola</em> spp.), red squirrel (<em>Sciurus vulgaris</em>), coypu (<em>Myocastor coypus</em>)</td>
</tr>
</tbody>
</table>
Some wild birds of Lombardy and Emilia-Romagna (mentioned in this presentation…)

<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anseriformes</td>
<td>mallard (<em>Anas platyrhynchos</em>), teal (<em>Anas crecca</em>), ducks (<em>Anas</em> spp.), swan (<em>Cygnus olor</em>)</td>
</tr>
<tr>
<td>Charadriiformes</td>
<td>gulls (<em>Larus</em> spp.), waders</td>
</tr>
<tr>
<td>Galliformes:</td>
<td>partridge (<em>Alectoris</em> spp.), quail (<em>Coturnix coturnix</em>)</td>
</tr>
<tr>
<td><em>Phasianidae</em></td>
<td></td>
</tr>
<tr>
<td>Passeriformes:</td>
<td>hooded crow (<em>Corvus corone cornix</em>), jackdaw (<em>Corvus monedula</em>), jay (<em>Garrulus glandarius</em>), magpie (<em>Pica pica</em>)</td>
</tr>
</tbody>
</table>
Some diseases of wildlife with zoonotic relevance

<table>
<thead>
<tr>
<th>BACTERIAL</th>
<th>VIRAL</th>
<th>PARASITIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>Rabies</td>
<td>Trichinellosis</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>Avian influenza</td>
<td>Echinococcosis</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>West Nile Disease</td>
<td>Toxoplasmosis</td>
</tr>
<tr>
<td>Salmonellosis</td>
<td>Aujeszky’s Disease</td>
<td></td>
</tr>
<tr>
<td>Tularemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lyme borreliosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlamydiosis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Tuberculosis (TB)

- A worldwide transmittable disease that affects a wide range of domestic and wild animals, as well as humans
- Affected wildlife may show no clinical signs
- Occurrence of TB in wildlife may directly affect the health of wild animal populations, as well eradication plans for TB in livestock and human health
Tuberculosis (TB)

Infection in wildlife is due to different species, including

- *Mycobacterium tuberculosis* complex
  (includes *M. bovis*, *M. caprae*, *M. microti*)

- *Mycobacterium avium*
Tuberculosis (TB)

Wildlife harbouring *M. tuberculosis* can act as

- **maintenance host**
  - when its infection persists through horizontal transmission between individuals in the absence of any other source
  - e.g., badger (UK), white-tailed deer (US), possum (NZ), African buffalo (Africa)

- **spill-over host**
  - when its infection occurs sporadically or persists if a maintenance host is present
## TB in wild boar

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive/Total</th>
<th>M. avium</th>
<th>M. terrae</th>
<th>M. t. complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>2/5</td>
<td>M. avium (1)</td>
<td>M. terrae (1)</td>
<td>M. t. complex (1)</td>
</tr>
<tr>
<td>2005</td>
<td>7/11</td>
<td>M. t. complex</td>
<td></td>
<td>M. t. complex (1)</td>
</tr>
<tr>
<td>2006</td>
<td>about 900 examined</td>
<td></td>
<td></td>
<td>M. microti (16)</td>
</tr>
<tr>
<td>2007</td>
<td>about 900 examined</td>
<td></td>
<td></td>
<td>M. microti (9)</td>
</tr>
<tr>
<td>2008</td>
<td>about 900 examined</td>
<td></td>
<td></td>
<td>M. microti (40)</td>
</tr>
</tbody>
</table>

- **2004**: 2/5
  - M. avium (1)
  - M. terrae (1)
- **2005**: 7/11
  - M. t. complex
- **2006**: about 900 examined
  - 59 with lesions
  - M. microti (16)
  - M. bovis (3)
- **2007**: about 900 examined
  - 63 with lesions
  - M. microti (9)
  - M. avium (2)
  - M. bovis (2)
- **2008**: about 900 examined
  - 59 with lesions
  - M. microti (40)
TB laboratory analyses

Macroscopic inspection of retropharyngeal and submandibular lymphnodes

When TB-like lesions are detected (small rounded necrotic, often calcified foci), samples undergo histological examination for confirmation, and both:

- culture in solid media and liquid culture system
- PCR directly from samples’ extracts, targeting IS6110
Cultured mycobacteria are identified by molecular and bacteriological tests. PCR-positive and culture-negative samples are also examined with a PCR targeting another gene (\textit{gyrB}), which allows to confirm the specificity of the IS6110 PCR.

Typing of mycobacteria is carried out with spoligotyping, a method based on PCR amplification of a highly polymorphic direct repeat locus in the \textit{M. tuberculosis} genome.
TB in red deer and roe deer

<table>
<thead>
<tr>
<th>Species</th>
<th>2007 positive/total</th>
<th>2008 positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red deer</td>
<td>9/109</td>
<td>0/1</td>
</tr>
<tr>
<td>M. microti (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. bovis (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. avium (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roe deer</td>
<td>1/38</td>
<td>0/82</td>
</tr>
<tr>
<td>M. microti (1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IZSLER, Sezione di Pavia, Italy Game Meat Hygiene in Focus, Brno, Czech Republic, 18-19 June 2009
TB in wildlife
some comments on the situation in Lombardy

• In Lombardy (differently from other Italian regions), it is **unlikely** that wild boar may act as a **reservoir** for bovine tuberculosis

• Genetic profiles of the few strains of *M. bovis* recovered **from wild boar** are **different** from those of strains isolated **from cattle** reared in the same area
TB in wildlife
some comments on the situation in Lombardy

• Until now, *M. microti* has been the most prevalent mycobacterial species detected in the wild boar in Lombardy

• Limited data are available for the red deer and roe deer, where both *M. microti* and *M. bovis* have been detected
Brucellosis in wildlife

- A worldwide disease that affects a wide range of domestic and wild animals, inducing abortion and reproductive disease (e.g., orchitis)
- Humans get infected from animal reservoirs, including wildlife, and contaminated animal products such as milk and cheese
- Occurrence of brucellosis in wildlife may affect eradication plans in livestock and human health
Brucellosis in wildlife

Wildlife may be infected with

- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
  biotype 1 (wild boar), biotype 2 (European hare, wild boar)

Brucellosis may occur as a spillover of infection from domestic animals or a sustainable infection that may pose a threat to livestock where eradication schemes are implemented.
## Brucellosis

<table>
<thead>
<tr>
<th>Species</th>
<th>2004 positive/total</th>
<th>2005 positive/total</th>
<th>2006 positive/total</th>
<th>2007 positive/total</th>
<th>2008 positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild boar</td>
<td>0/170</td>
<td>0/152</td>
<td>0/162</td>
<td>0/433</td>
<td>0/115</td>
</tr>
<tr>
<td>Chamois</td>
<td>0/245</td>
<td>--</td>
<td>0/191</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Red deer</td>
<td>0/8</td>
<td>--</td>
<td>0/52</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>Roe deer</td>
<td>0/1</td>
<td>0/224</td>
<td>0/24</td>
<td>0/79</td>
<td>0/13</td>
</tr>
<tr>
<td>European hare</td>
<td>0/271</td>
<td>0/14</td>
<td>0/109</td>
<td>0/93</td>
<td>2/195</td>
</tr>
</tbody>
</table>
Leptospirosis

- Bacterial disease due to spirochaetes that are shed in urine of wild and domestic animals and thus may contaminate water and soil
- In urban and rural environments, rodents are considered to be the most important reservoir of leptospirosis
Leptospirosis

Typical lesions due to leptospirosis in animals are chronic interstitial nephritis and renal fibrosis.
## Leptospirosis

<table>
<thead>
<tr>
<th>Animal</th>
<th>2004 positive/total</th>
<th>2005 positive/total</th>
<th>2006 positive/total</th>
<th>2007 positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild boar</td>
<td>20/337</td>
<td>49/278</td>
<td>36/542</td>
<td>2/81 (PCR)</td>
</tr>
<tr>
<td>Chamois</td>
<td>0/12</td>
<td>0/47</td>
<td>1/45</td>
<td>0/2</td>
</tr>
<tr>
<td>Red deer</td>
<td>--</td>
<td>--</td>
<td>1/45</td>
<td>0/6</td>
</tr>
<tr>
<td>Roe deer</td>
<td>--</td>
<td>1/43</td>
<td>0/24</td>
<td>0/2</td>
</tr>
<tr>
<td>European hare</td>
<td>0/9</td>
<td>0/12</td>
<td>1/84</td>
<td>0/81</td>
</tr>
<tr>
<td>Coypu</td>
<td>5/48</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

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Salmonellosis

<table>
<thead>
<tr>
<th>Species</th>
<th>2006 positive/total</th>
<th>2007 positive/total</th>
<th>2008 positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red deer</td>
<td>--</td>
<td>1/67</td>
<td>--</td>
</tr>
<tr>
<td>Wild boar</td>
<td>92/311</td>
<td>123/666</td>
<td>79/696</td>
</tr>
<tr>
<td>Red fox</td>
<td>0/17</td>
<td>2/17</td>
<td>--</td>
</tr>
</tbody>
</table>

- *Salmonella anatum* in hooded crow
- *Salmonella typhimurium* in feral pigeons
- *Salmonella enterica* serotype 4,12 in heron
Tularemia

- Bacterial disease due to *Francisella tularensis* (type A and B)
- Arthropod-borne (ticks, mosquitoes, tabanids) as well as via direct transmission
- Also waterborne!
- Rodents and lagomorphs mostly involved
- Bioterrorism agent cat. A
Tularemia

- Oltrepo’ Pavese has been an endemic area since 1960
- Recent waterborne outbreaks
- IZSLER, Sezione di Pavia is the National Reference Laboratory for Italy
- Direct diagnosis: isolation, PCR
- Indirect diagnosis: agglutination test

Francisella tularensis
Gram stain
Tularemia in the European hare
- splenomegaly -
## Tularemia

<table>
<thead>
<tr>
<th>Species</th>
<th>2004 positive/total</th>
<th>2005 positive/total</th>
<th>2006 positive/total</th>
<th>2007 positive/total</th>
<th>2008 positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>European hare</td>
<td>0/329</td>
<td>0/19</td>
<td>0/118</td>
<td>0/130</td>
<td>1/453</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3/121 (PCR)</td>
<td></td>
<td>3/57 (PCR)</td>
</tr>
<tr>
<td>Roe deer</td>
<td>--</td>
<td>0/248</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Coypu</td>
<td>0/15</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Lyme borreliosis

- Bacterial tick-borne disease, main vector in Italy is the hard tick *Ixodes ricinus*
- *Borrelia burgdorferi, B. afzelii, B. garinii*
- Mostly asymptomatic in animals
- Dermatological disease, arthritis and neurological disease in humans
- It has been found in several Italian regions: northeast Italy (Friuli Venezia Giulia, Veneto, Trento), Ligurian coast, Emilia-Romagna
## Lyme borreliosis

<table>
<thead>
<tr>
<th>Species</th>
<th>2006 positive/total</th>
<th>2007 positive/total</th>
<th>2008 positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roe deer</td>
<td>47/267</td>
<td>29/423</td>
<td>3/12</td>
</tr>
<tr>
<td>Chamois</td>
<td>8/47</td>
<td>1/330</td>
<td>--</td>
</tr>
<tr>
<td>European hare</td>
<td>1/10</td>
<td>0/26</td>
<td>--</td>
</tr>
<tr>
<td>Ibex</td>
<td>--</td>
<td>0/1</td>
<td>--</td>
</tr>
<tr>
<td>Wild boar</td>
<td>--</td>
<td>0/37</td>
<td>--</td>
</tr>
</tbody>
</table>
Paratuberculosis

- Bacterial disease, *Mycobacterium avium* subsp. *paratuberculosis*
- Suspected link between *paratuberculosis* and *Crohn’s disease* in humans, not proven!
- Medium to high prevalences in bovine herds of some parts of Lombardy and Emilia-Romagna, but control plans ongoing
- Also affecting small ruminants
<table>
<thead>
<tr>
<th>Species</th>
<th>2004 positive/total</th>
<th>2005 positive/total</th>
<th>2006 positive/total</th>
<th>2007 positive/total</th>
<th>2008 positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamois</td>
<td>0/2</td>
<td>--</td>
<td>0/261</td>
<td>0/327</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0/36 (PCR)</td>
<td>0/46 (PCR)</td>
<td></td>
</tr>
<tr>
<td>Red deer</td>
<td>1/17</td>
<td>0/10</td>
<td>0/9</td>
<td>0/19</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>0/9 (PCR)</td>
<td>0/10 (PCR)</td>
<td>1/25 (PCR)</td>
<td>2/11 (PCR)</td>
<td>5/6 (PCR)</td>
</tr>
<tr>
<td>Roe deer</td>
<td>0/3</td>
<td>0/204</td>
<td>--</td>
<td>1/417</td>
<td>0/322</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2/67 (PCR)</td>
<td>6/30 (PCR)</td>
</tr>
</tbody>
</table>
Chlamydiases

- Bacterial disease with several species involved, also novel organisms detected
- Pneumonia in chamois
- Abortion in roe deer
- Conjunctivitis in ibex
- No clinical signs, partridge
- No clinical signs? in wild birds
Other bacterial diseases

- *Yersinia enterocolitica*
  - detected in 9/107 roe deers affected with diarrhea
  - no other organism detected

- *Pasteurella* sp. and *Mannheimia* sp.
  - detected in European hare, chamois, red deer affected with pneumonia
Other diseases

Chronic wasting disease

- never detected in the red deer

Positive/tested

0/256 (2006)
0/247 (2007)
Toxoplasmosis

- Parasitic protozoal disease
- Final hosts are felids, intermediate hosts are warm-blooded animals (mammals and birds)

Detected in:
- wild boar (Ab) positive/tested: 82/210 (2007)
- European hare (direct detection)
Echinococcosis

*Echinococcus multilocularis*, the agent of alveolar echinococcosis/hydatidosis, has never been detected in Lombardy*, but foci of infection in red foxes have been reported in Trentino-Alto Adige, a region to the east of Lombardy, since 2002

* A recent (2005) survey on 102 foxes in Lombardy found no evidence of the parasite
Trichinellosis

- Parasitic disease
- Main hosts are omnivores and carnivores, scavengers
- 8 species, 3 genotypes
- Most species infect mammals
- 1 species found also in birds, 2 other species also in reptiles
# Trichinellosis

<table>
<thead>
<tr>
<th>Species</th>
<th>2004 positive/total</th>
<th>2005 positive/total</th>
<th>2006 positive/total</th>
<th>2007 positive/total</th>
<th>2008 positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild boar</td>
<td>0/202</td>
<td>0/191</td>
<td>0/1196</td>
<td>11/6111</td>
<td>0/8952</td>
</tr>
<tr>
<td>Red fox</td>
<td>--</td>
<td>--</td>
<td>0/21</td>
<td>0/87</td>
<td>1/204</td>
</tr>
<tr>
<td>Corvids</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0/598</td>
</tr>
</tbody>
</table>
Rabies

- Viral disease (Rhabdovirus)
- Not detected in wildlife in Italy since 1997
- Virus found in a red fox in October 2008 in a north-eastern Italian region (Friuli Venezia Giulia)
- Positive animals up to now: 13 red foxes, 2 badgers, 1 roe deer
- No cases in Lombardy and Emilia-Romagna

<table>
<thead>
<tr>
<th>Species</th>
<th>2005 positive/total</th>
<th>2006 positive/total</th>
<th>2007 positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red fox</td>
<td>0/837</td>
<td>0/3824</td>
<td>0/635</td>
</tr>
</tbody>
</table>
Rabies
Detection sites of infected wildlife (2008-2009)
West Nile Virus disease

**Incidental hosts**
Humans, horses, and other animals

**Amplifying hosts**
Birds

**Vectors**
*Culex sp.*, *Aedes sp.*, *Ochlerotatus sp.*
West Nile Virus disease

- Viral disease (Flavivirus)
- October 2008: detection of WNV in 42/651 wild birds (magpie, hooded crows, jays, pigeons, gulls, cormorant) following the investigation of an outbreak of disease in horses affected with neurological signs
- Human patient involved with neurological signs as well

Avian Influenza

- Viral disease (Orthomyxovirus)
- Wild birds, mostly anseriformes (ducks) and charadriiiformes (gulls, waders), are reservoir of all H1-H15 subtypes of influenza viruses
- Surveillance in Lombardy and Emilia-Romagna focuses on the Po river delta, and the Valli di Comacchio
- Detection of Low Pathogenicity Avian Influenza (LPAI) viruses in common teal and mallard (3/793)
Some comments on the presented data

- **Most diseases of wildlife** in Lombardy and Emilia-Romagna have occurred through the years at **low or moderate levels**, with some notable exceptions (e.g., *Leptospira*, mycobacterial infections, *Salmonella*)

- The **prevalent local wildlife host** for some disease has been **recognized or confirmed** (e.g., roe deer for Lyme borreliosis, wild boar for mycobacterial infections and leptospirosis)
Some comments on the presented data

- Of particular relevance, a network has been consolidated through the years among the veterinary services, public administration and the hunters, which is crucial as to the investigation of wildlife diseases in the area.

- This led to an increased number of samples being submitted to the laboratory and to additional tests being offered.
Acknowledgements

• Alessandra Gaffuri, IZSLER Sezione di Bergamo
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• Maria Grazia Zanoni, IZSLER Sezione di Brescia
• Ana Moreno Martin, IZSLER Sezione di Brescia
Time to go…

Thank you for your attention!