International Conference “Game Meat Hygiene in Focus”
University of Veterinary and Pharmaceutical Science Brno, Czech Republic
June 18th-19th, 2009

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

Simone Magnino, DVM, Dipl. ECVPH
Istituto Zooprofilattico Sperimentale
della Lombardia e dell’Emilia Romagna “Bruno Ubertini”
Sezione Diagnostica di Pavia
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

Relevance of the monitoring of wildlife disease

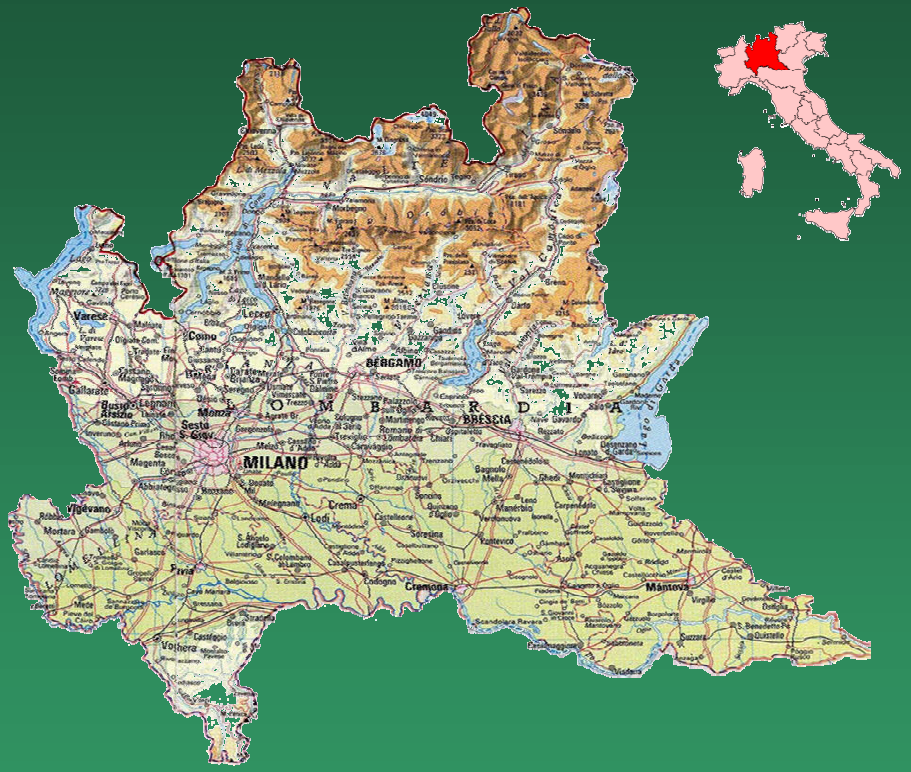
Wildlife health is a cause for common concern for different stakeholders, such as

- 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 km^2
- Population: 9,714,640 (09/2008)

- Area: 22,123 km^2
- Population: 4,293,825 (12/2008)

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna
- one in a network of regional veterinary laboratories -

Istituto Zooprofilattico Sperimentale

- del Piemonte, Liguria e Valle d'Aosta
- della Lombardia e Emilia Romagna (IZSLER)
- delle Venezie
- dell'Umbria e Marche
- del Lazio e Toscana
- del Mezzogiorno
- dell'Abruzzo e Molise
- della Puglia e Basilicata
- della Sicilia
- della Sardegna

IZSLER

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…)

Ruminants	roe deer (*Capreolus capreolus*), alpine chamois (*Rupicapra rupicapra*), red deer (*Cervus elaphus*), ibex (*Capra ibex*)
Canids	red fox (*Vulpes vulpes*)
Suids	wild boar (*Sus scrofa*)
Mustelids	badger (*Meles meles*), beech marten (*Martes foina*), pine marten (*Martes martes*), stoat (*Mustela erminea*), weasel (*Mustela nivalis*)
Lagomorphs	European hare (*Lepus europaeus*), mountain hare (*Lepus timidus*), wild rabbit (*Oryctolagus cuniculus*)
Rodents	mice (*Apodemus* spp., …), voles (*Microtus* spp., *Arvicola* spp.), red squirrel (*Sciurus vulgaris*), coypu (*Myocastor coypus*)

Some wild birds of Lombardy and Emilia-Romagna

(mentioned in this presentation…)

Anseriformes	mallard (*Anas platyrhynchos*), teal (*Anas crecca*), ducks (*Anas* spp.), swan (*Cygnus olor*)
Charadriiformes	gulls (*Larus* spp.), waders
Galliformes: *Phasianidae*	partridge (*Alectoris* spp.), quail (*Coturnix coturnix*)
Passeriformes: *Corvidae*	hooded crow (*Corvus corone cornix*), jackdaw (*Corvus monedula*), jay (*Garrulus glandarius*), magpie (*Pica pica*)

Some diseases of wildlife with zoonotic relevance

BACTERIAL

- Tuberculosis
- Brucellosis
- Leptospirosis
- Salmonellosis
- Tularemia
- Lyme borreliosis
- Chlamydiosis

VIRAL

- Rabies
- Avian influenza
- West Nile Disease
- Aujeszky's Disease

PARASITIC

- Trichinellosis
- Echinococcosis
- Toxoplasmosis

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 tasnsmission 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 occur sporadically or persists if a maintenance host is present

TB in wild boar

2004 positive/total	2005 positive/total	2006 positive/total	2007 positive/total	2008 positive/total
2/5 *M. avium* (1) *M. terrae* (1) 2/14 *M. t. complex* (1) *M. avium* (1)	7/11 *M. t. complex*	about 900 examined 59 with lesions 19/59 *M. microti* (16) *M. bovis* (3)	about 900 examined 63 with lesions 23/63 *M. microti* (9) *M. avium* (2) *M. bovis* (2)	about 900 examined 59 with lesions 40/59 *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

TB laboratory analyses

Cultured mycobacteria are identified by molecular and bacteriological tests

PCR-positive and culture-negative samples are also examined with a PCR targeting another gene (*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 *M. tuberculosis* genome

TB in red deer and roe deer

Species	2007 positive/total	2008 positive/total
Red deer	9/109 *M. microti* (2) *M. bovis* (2) *M. avium* (2)	0/1
Roe deer	1/38 *M. microti* (1)	0/82

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

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

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

	2004 positive/total	2005 positive/total	2006 positive/total	2007 positive/total
Wild boar	20/337	49/278	36/542 10/115 (PCR)	2/81 2/107 (PCR)
Chamois	--	--	0/47	0/2
Red deer	--	--	1/45	0/6
Roe deer	--	1/43	0/24	0/2
European hare	0/9	0/12	1/84	0/81
Coypu	5/48	--	--	--

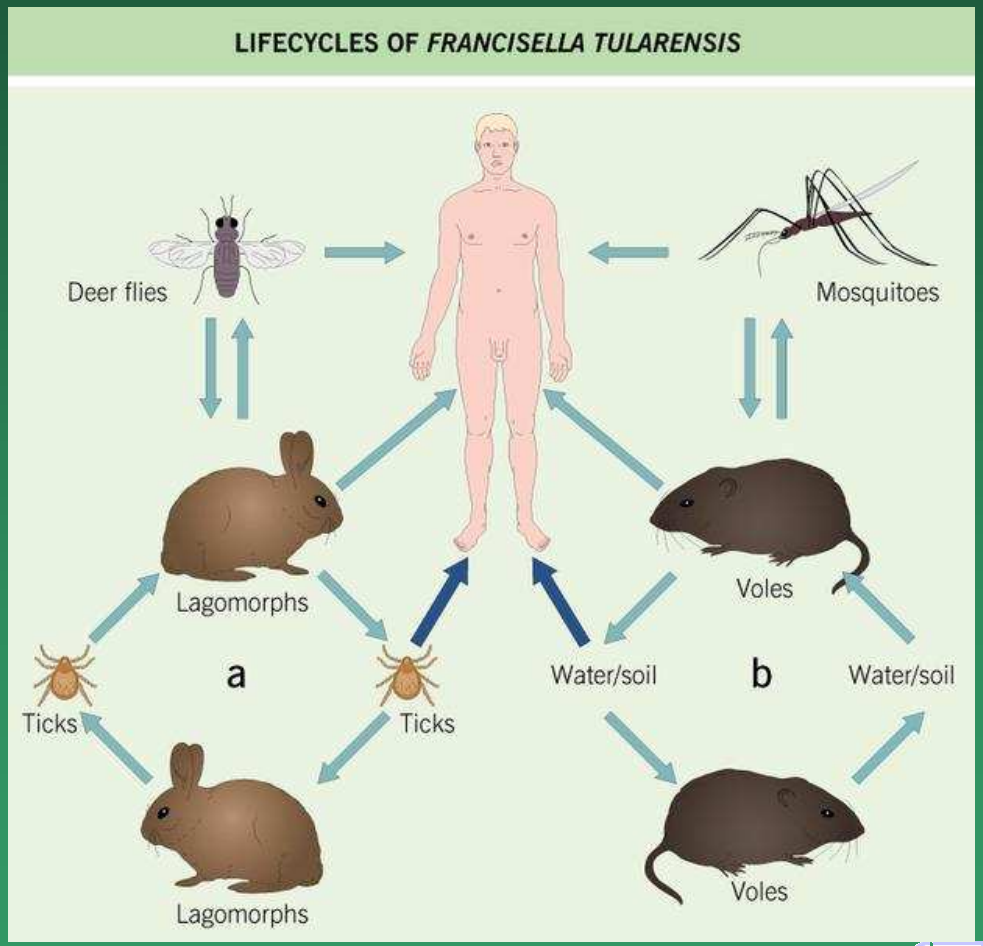
Salmonellosis

Species	2006 positive/total	2007 positive/total	2008 positive/total
Red deer	--	1/67	--
Wild boar	92/311	123/666	79/696
Red fox	0/17	2/17	--

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

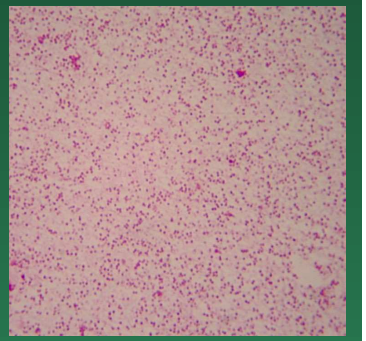
Tularemia

- 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

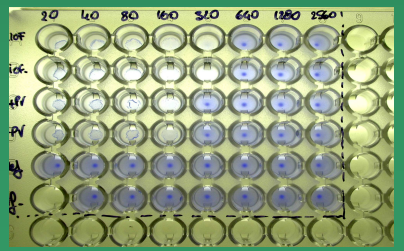


Tularemia

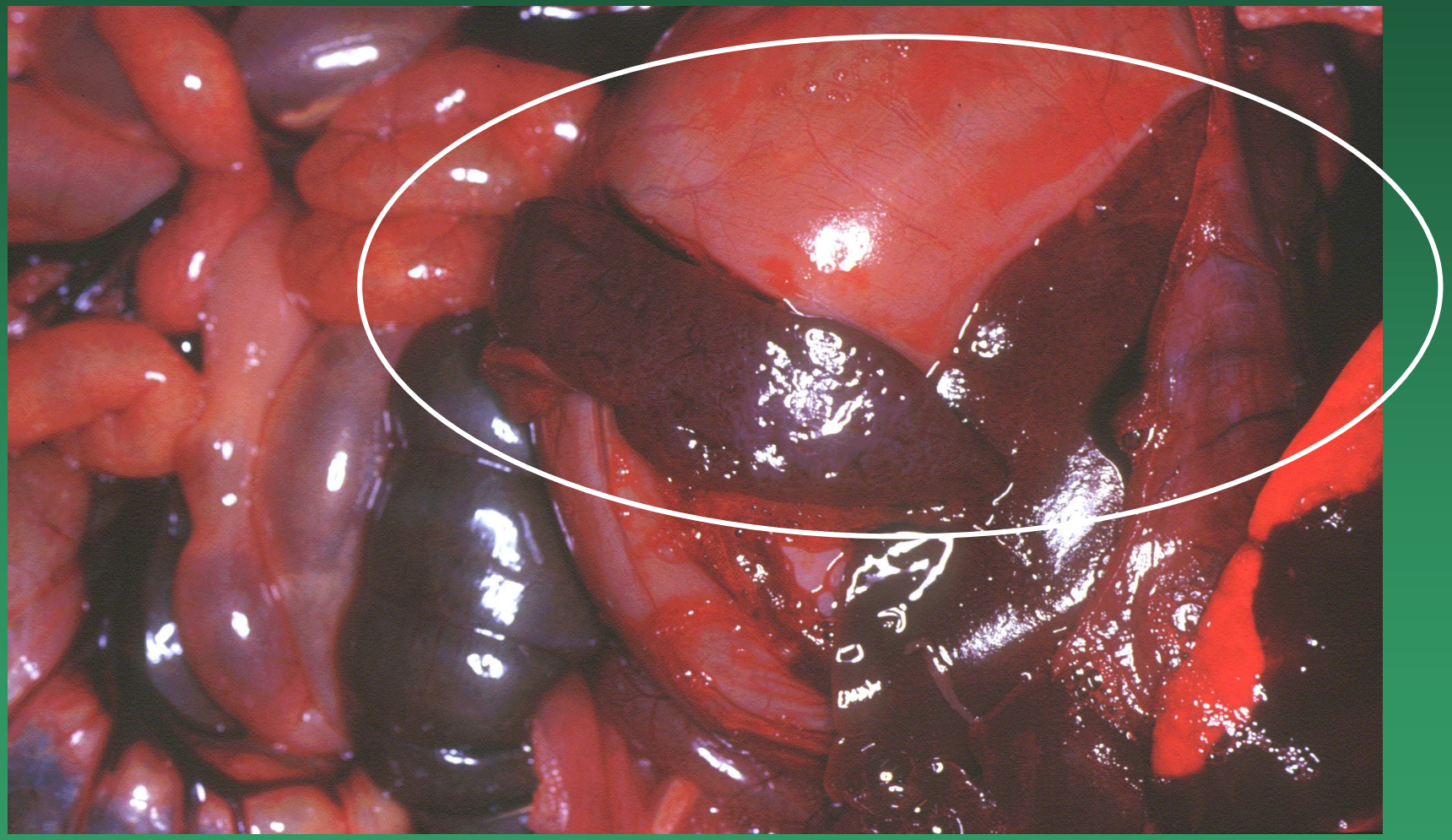
- Oltrepo' Pavese has been an endemic area since 1960
- Recent waterborne outbreaks
- IZSLER, Sezione di Pavia is the National Reference Laboratory for Italy
- Direct diagnosis: isolation, PCR
- Indirect diagnosis: agglutination test



Francisella tularensis
Gram stain



Tularemia in the European hare
- splenomegaly -



Tularemia

Species	2004 positive/total	2005 positive/total	2006 positive/total	2007 positive/total	2008 positive/total
European hare	0/329	0/19 3/121 (PCR)	0/118	0/130	1/453 3/57 (PCR)
Roe deer	--	0/248	--	--	--
Coypu	0/15	--	--	--	--

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, artrhitis 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

Species	2006 positive/total	2007 positive/total	2008 positive/total
Roe deer	47/267	29/423	3/12
Chamois	8/47	1/330	--
European hare	1/10	0/26	--
Ibex	--	0/1	--
Wild boar	--	0/37	--

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

Paratuberculosis

Species	2004 positive/total	2005 positive/total	2006 positive/total	2007 positive/total	2008 positive/total
Chamois	0/2	--	0/261 0/36 (PCR)	0/327 0/46 (PCR)	--
Red deer	1/17 0/9 (PCR)	0/10 0/10 (PCR)	0/9 1/25 (PCR)	0/19 2/11 (PCR)	1/10 5/6 (PCR)
Roe deer	0/3	0/204	--	1/417 2/67 (PCR)	0/322 6/30 (PCR)

Chlamydioses

- 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

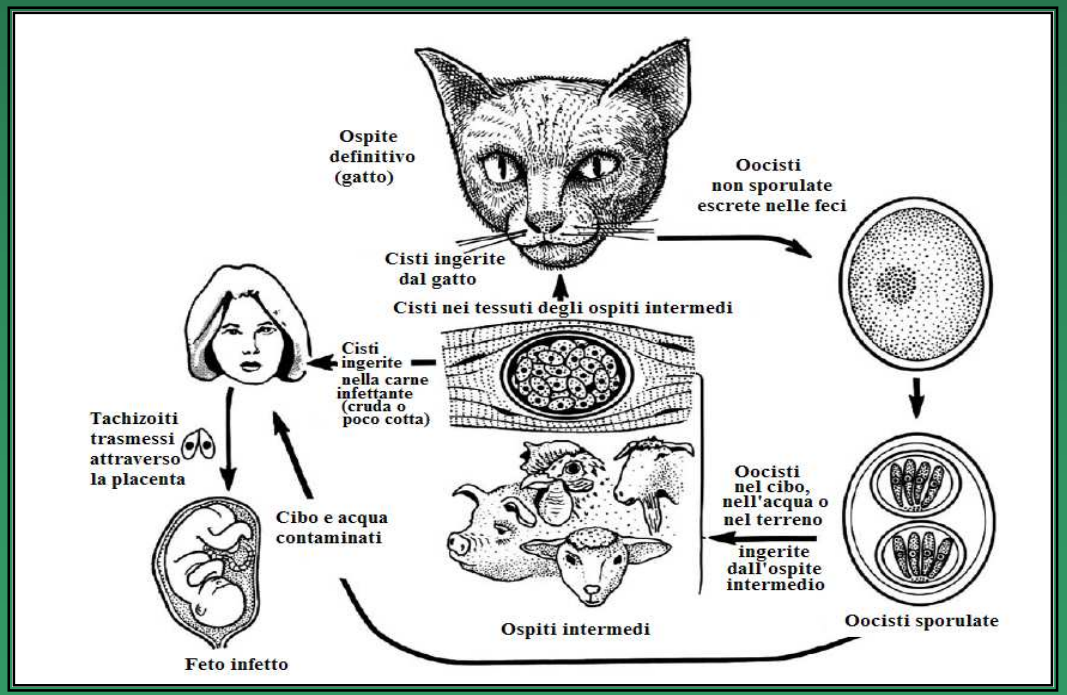
Positive/tested

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

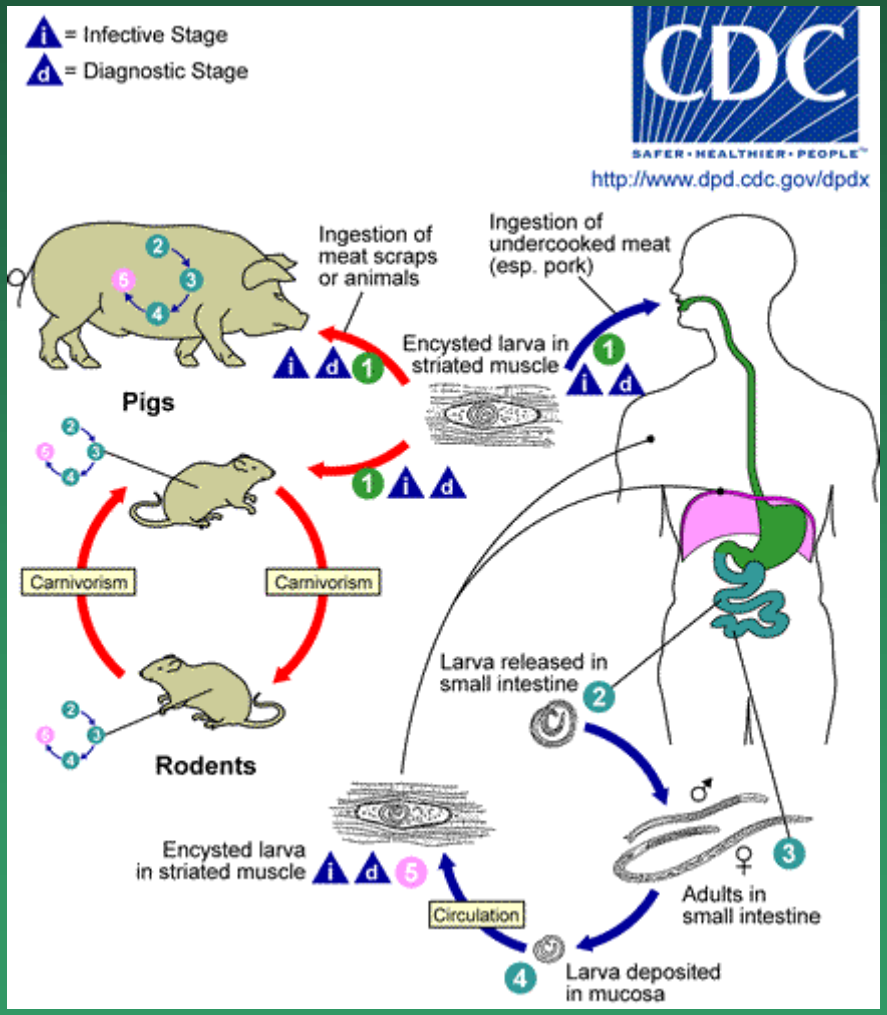
- wild boar (Ab) positive/tested: 82/210 (2007)
- European hare (direct detection)

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

Species	2004 positive/total	2005 positive/total	2006 positive/total	2007 positive/total	2008 positive/total
Wild boar	0/202	0/191	0/1196	11/6111	0/8952
Red fox	--	--	0/21	0/87	1/204
Corvids	--	--	--	--	0/598

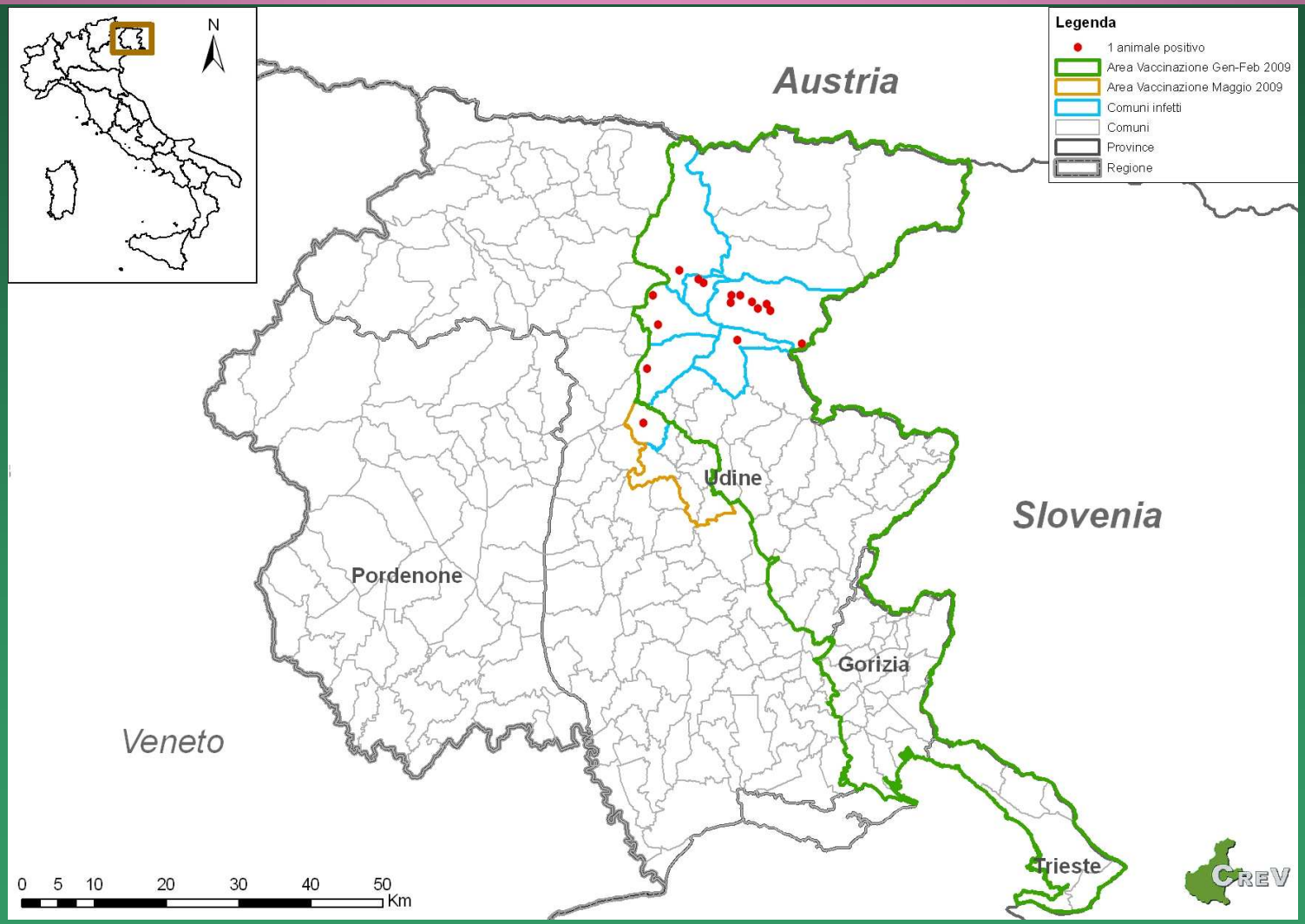
Rabies

Species	2005 positive/total	2006 positive/total	2007 positive/total
Red fox	0/837	0/3824	0/635

- 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

Rabies

Detection sites of infected wildlife (2008-2009)



West Nile Virus disease

Amplifying hosts

Birds

Vectors

Culex sp., Aedes sp., Ochlerotatus sp.

Incidental hosts

Humans, horses, and other animals

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

Macini *et al.* (2008). Detection of West Nile virus infection in horses, Italy, September 2008. *Eurosurveillance 13 (39),* 25 September 2008

IZSLER, Sezione di Pavia, Italy

Avian Influenza

- Viral disease (Orthomyxovirus)
- Wild birds, mostly anseriformes (ducks) and charadriiformes (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

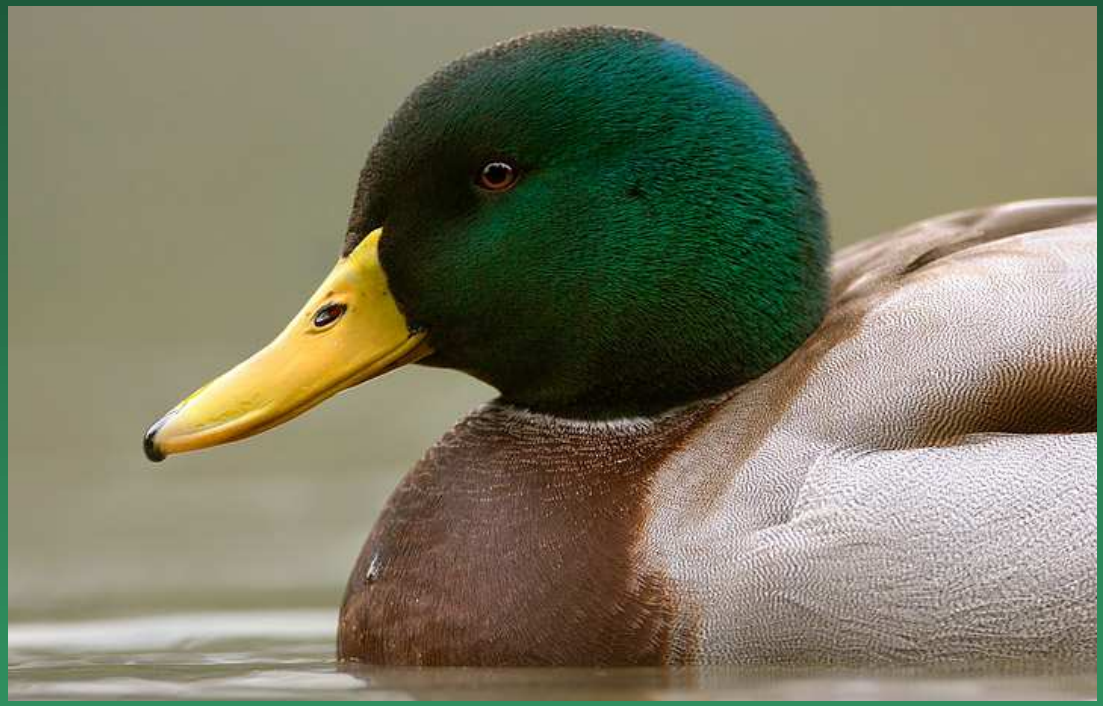
- Most diseases of wildlife in Lombardy and Emilia-Romagna have occurred through the years at low or moderate levels, with some notable exceptions (e.g., *Leptospira*, mycobacterial infections, *Salmonella*)
- The prevalent local wildlife host for some disease has been recognized or confirmed (e.g., 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
- Matteo Frasnelli, IZSLER Sezione di Ferrara
- Maria Grazia Zanoni, IZSLER Sezione di Brescia
- Ana Moreno Martin, IZSLER Sezione di Brescia

Time to go…

Thank you for your attention !