Erasmus MC

Viroscience lab

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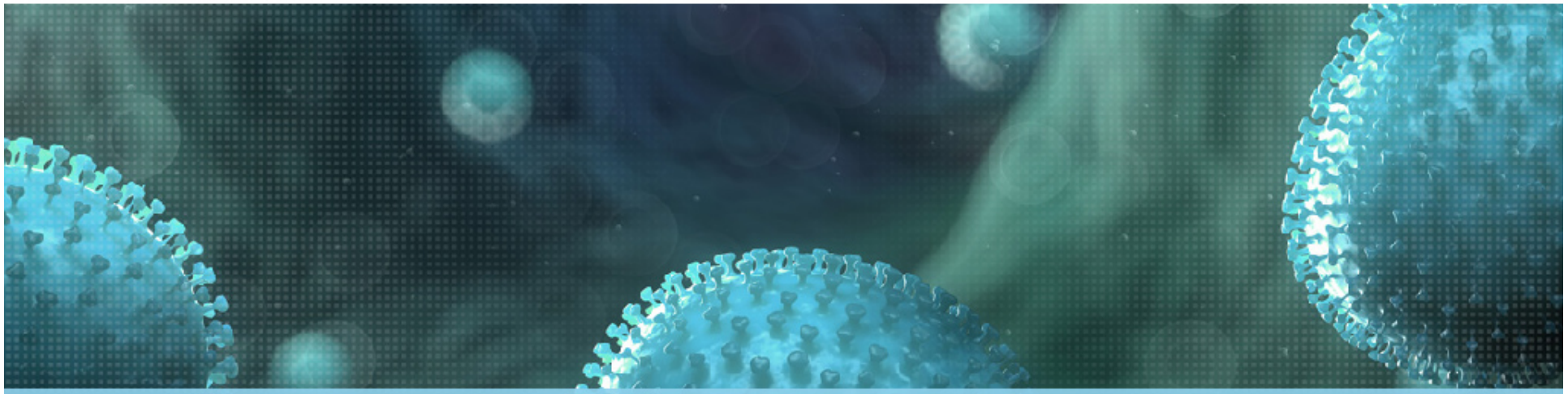


Harmonizing methods for sampling and diagnosing wildlife diseases

Thijs Kuiken

APHAEA meeting, Brescia, 27 June 2013

Pathogen surveillance in wild and domestic animals important for managing EIDs

- Increased frequency of emergence of infectious diseases in last 20 years
- Emerging infectious diseases have enormous impact on
  - Public health
  - Food supply
  - Economies
  - Environment
- Wild or domestic animals are main source of these infections
- Therefore, pathogen surveillance in animals is important

Impact of selected EIDs

Virus	Appeared in	Area	Reservoir/source	Impact
HIV-1	1981	Global	Chimpanzee	▪20 million human deaths ▪38 million people currently infected ▪US$ 5 billion for response in 2003
Nipah virus	1998	Malaysia, Singapore	Flying foxes	▪106 deaths of 276 human cases ▪>1 million pigs culled
West Nile virus	2001	North and Central America	Wild birds/ mosquitoes	▪683 deaths of 18,269 human cases ▪22,566 equine cases in USA ▪100 000s of wild birds
Avian influenza virus (H5N1)	1997	Southeast Asia	Poultry	▪62 deaths of 129 human cases ▪Nearly 140 million poultry dead ▪Direct economic costs > US$ 10 billion

Current animal pathogen surveillance: national

- Domestic
  - Department of agriculture
  - Quality variable among countries
- Wildlife
  - Only in some countries
  - Limited scope
  - No clear reporting conventions

Current animal pathogen surveillance: international

- World Organization for Animal Health (OIE)
  - Reporting of pathogens affecting trade and/or public health
  - International Early Warning System (immediate reporting)
  - International Monitoring System (semi-annual to annual)
  - Wildlife Disease Working group (semi-annual)

- United Nations Food and Agricultural Organization (FAO)
  - Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases, or EMPRES

- World Health Organization (WHO)
  - Global Outbreak Alert and Response Network, or GOARN

- International Society for Infectious Diseases
  - Program for Monitoring Emerging Diseases (ProMED; www.promedmail.org)

Current system provides insufficient level of vigilance

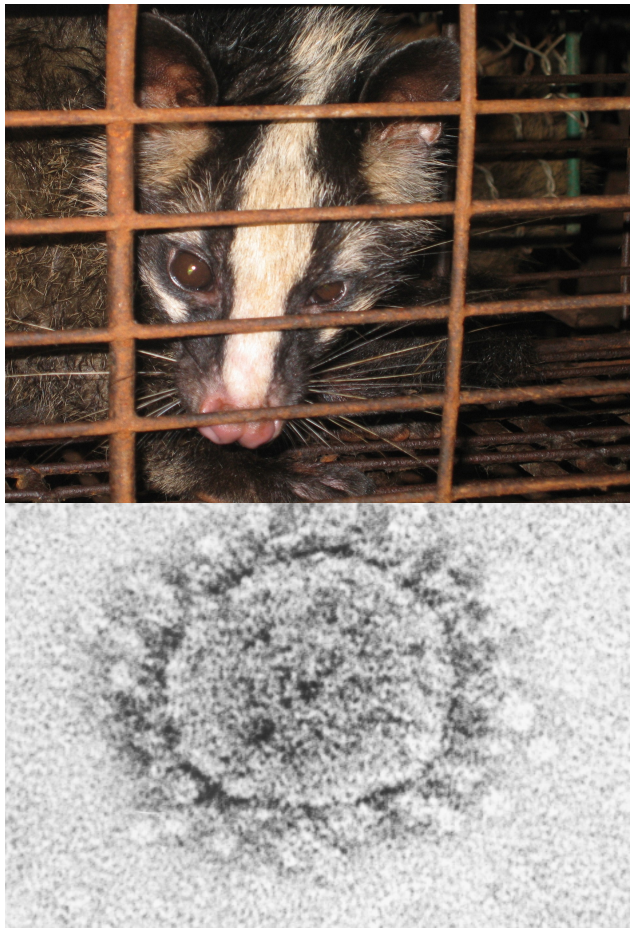
1. Pathogen surveillance in domestic animals generally confined to pathogens with known economic impacts

2. Pathogen surveillance in wild animals less intensive to nonexistent

3. Lack of integration among pathogen surveillance systems in humans, domestic animals, and wildlife

Problems with current surveillance: SARS-associated coronavirus in Asia

- November 2002, Guangdong, China
  - New disease, SARS, appeared in humans
  - SARS-CoV identified as cause
- Initially transmitted to humans by wild animals sold as exotic food
- Source of these wild animals (both from China and other countries)
  - Game farms
  - Wild-caught
- Absence of
  - Animal virus surveillance data
  - Archived sera or tissue samples
- Not possible to retrospectively trace source of virus (in time or space)



Problems with current surveillance: Avian influenza virus (H7N7) in Europe

- February 2003, Netherlands
  - Epidemic of highly pathogenic avian influenza
  - Caused by H7N7 virus

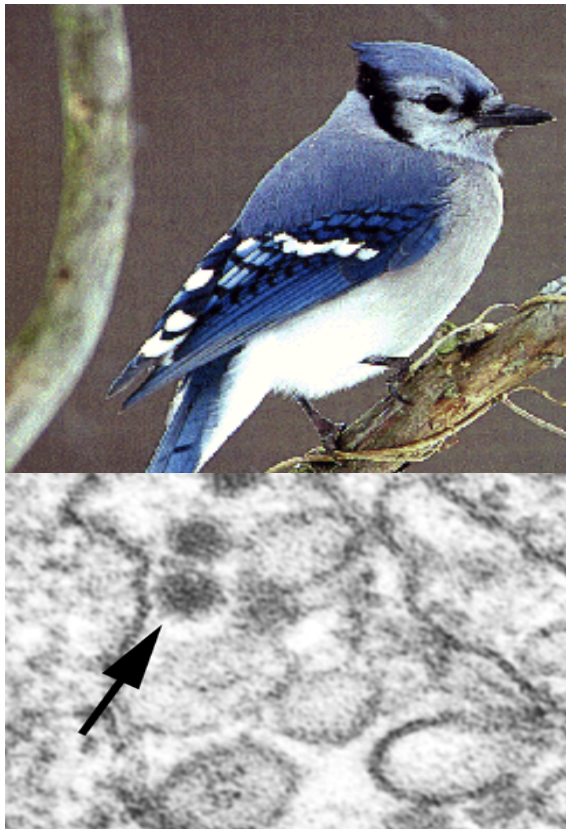
- Sequence of events
  - Likely originated from low pathogenic H7N7 virus in free-living ducks
  - Evolved into high pathogenic variant after entering poultry farms

- Retrospective serological screening
  - Egg production decrease on two poultry farms four months before epidemic
  - Respiratory problems on turkey farm two months before epidemic
  - Antibody to H7 influenza virus

- H7 influenza virus affecting Dutch poultry industry several months before major epidemic, but not recognized as such

Problems with current surveillance: West Nile virus in North America

- August 1999, New York
  - Cluster of 8 human patients with encephalitis
  - Initially diagnosed as St Louis encephalitis virus (flavivirus)
- Unusual mortality of wild and captive birds in Bronx Zoo
  - St Louis encephalitis virus doesn't kill birds, so other pathogen
  - Diagnosed as West Nile virus, not found before in North America
- Human cases subsequently also diagnosed as West Nile virus
- Had wild bird mortality not been investigated, discovery of WNV in North America may have been delayed

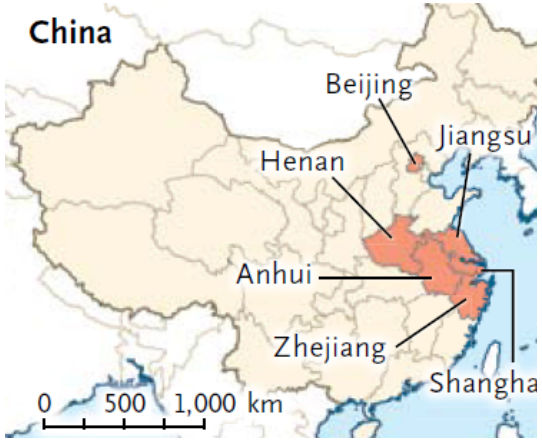


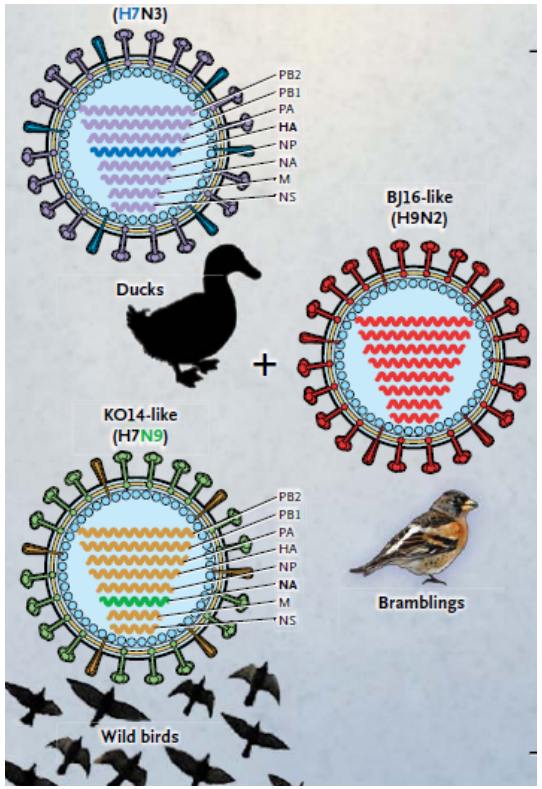
Problems with current surveillance: MERS-CoV in the Middle East

- October 2012, Saudi Arabia *(Zaki 2013, NEJM)*
  - Novel coronavirus in man with acute pneumonia
  - Identified as HCoV-EMC
- 17 June 2013 *(www.who.int)*
  - 64 confirmed infections
  - 38 deaths
- Evidence for zoonotic transmission *(Annan 2013 EID)*
  - Viruses from Nycteris bats Ghana
  - Viruses from Pipistrellus bats Europe
  - Patient contact with camels/goats?
- Animal source of virus not yet determined

Problems with current surveillance: Avian influenza virus H7N9 in China

- March 2013, east China *(Gao 2013, NEJM)*
  - 3 human patients diagnosed with H7N9
  - Reassortant
    - HA from H7N3 duck
    - NA from H7N9 unspecified wild bird
    - Internal genes H9N2 brambling
- 30 May 2013 *(www.who.int)*
  - 132 confirmed infections
  - 37 deaths
- Evidence for zoonotic transmission
  - 76% cases contact live chickens *(Li 2013, NEJM*
  - 20 of 970 environmental samples from live poultry markets positive for H7N9 *(Shi 2013, Chin Sci Bull)*
- To date, animal species from which H7N9 originated not determined





What is the solution for wildlife disease surveillance?

- Extremes for improved wildlife surveillance system
  - Rapid response team only, don't fix surveillance system
    - E.g., WHO-sponsored SARS Aetiology Study Group
    - 12 laboratories from different countries
  - Perfect surveillance system
    - E.g. (for one host and one pathogen), WHO Global Influenza Surveillance Network
    - 4 collaborating centres and 112 national centres in 83 countries

- Compromise
  - Fix largest gaps in surveillance system
  - Allow sufficient flexibility (personnel, finances) to respond to unexpected outbreaks

What is the situation for wildlife disease surveillance in Europe?

Rev. sci. tech. Off. int. Epiz., 1995, **14** (3), 819-830

Surveillance of wild animal diseases in Europe

F.A. LEIGHTON *

Level of wildlife disease surveillance in Europe

(Leighton 1995, Rev Sci Tech)

1. **Comprehensive general surveillance**

   Programmes which cover the entire country and are comprehensive with respect to species of mammals and birds examined and types of diseases assessed

2. **Partial general surveillance**

   Wide range of programmes including detection, diagnosis and management of disease-related information, but restricted in various ways (e.g. to certain geographical regions or groups of species)

3. **No general surveillance**

   Absence of a programme of general wild animal disease surveillance (in all cases, however, there is some degree of surveillance for a few specified diseases, e.g. rabies, bovine tuberculosis or classical swine fever [hog cholera], and wild animals are included in such surveillance to some degree)

General characteristics of wild animal disease surveillance in European countries in 1993-1994

Country	Comprehensive general surveillance [a]	Partial general surveillance [b]	No general surveillance [c]	Records covering 10 years or more (date of earliest record)	Computerised records (date computerisation was started)
Austria		×		1978	
Belgium			×		
Croatia			×		
Czech Republic			× [d]	1980	
Denmark	×			1934	
Estonia			×		
Finland	×			1930	1990
France		×			1986
Germany		×		1953 [e]	1994 [e]
Greece		×			
Hungary		×		1977	1984
Ireland			×		
Italy		×			
Latvia			×		
Lithuania			×		
Luxemburg			×		
The Netherlands			×		
Norway	×			1960/1891	1985
Poland			×		
Portugal			×		
Russia			× [f]		
Serbia and Montenegro		×		1957	
Slovenia		×		1953	
Spain		×			
Sweden	×			1945	1986
Switzerland		×		1950 [g]	1994 [g]
				1984 [h]	1984 [h]
United Kingdom		×		1975 [i]	1975 [i]

Establishment of the European section of the Wildlife Disease Association, 1993

"A meeting of interested individuals was held at the Zoological Society of London, 3 and 4 February 1993 …

A formal application to WDA to create a European Section was generated and an interim Board was organized …

Critical issues having been met, a motion was made (Fairbrother/ Botzler) and approved regarding establishment of a European Section."

From: Minutes of the 1993 council meeting of the WDA, held on 8 August, 1993, in Guelph, Ontario.

EWDA network for wildlife health surveillance in Europe

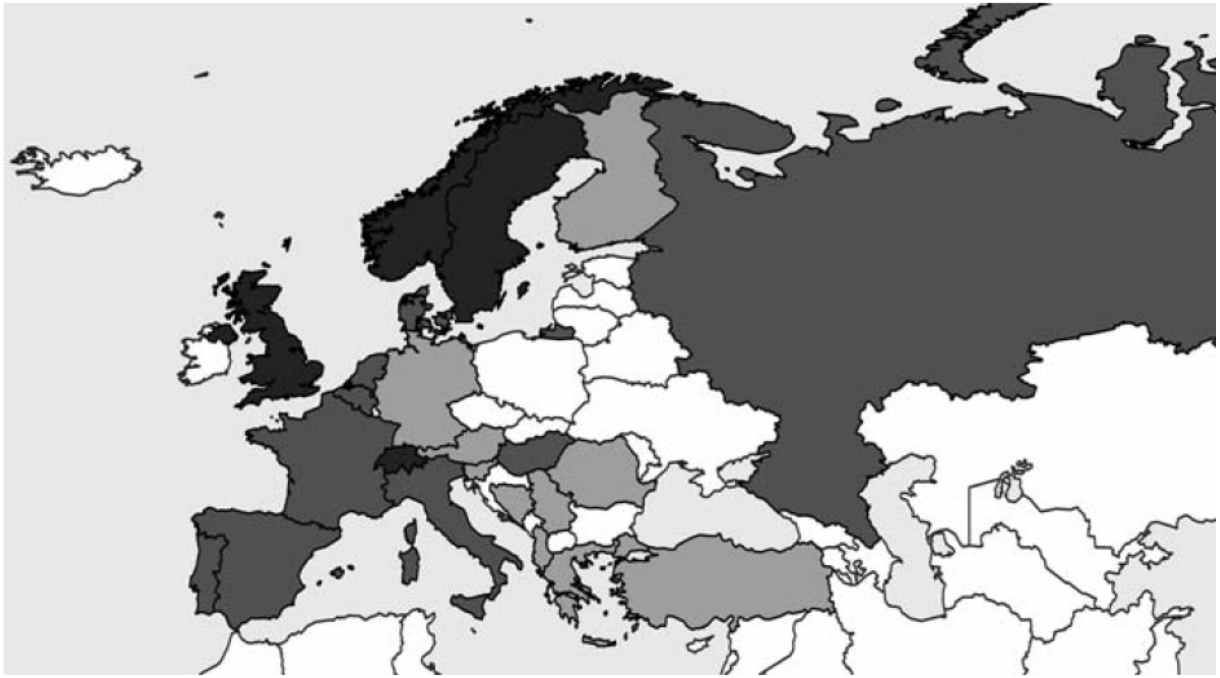
Inaugural meeting in Brussels on 15 October 2009

Country summaries provided by

- Kastriot Korro (Albania)
- Landry Riba (Andorra)
- Gabrielle Stalder (Austria)
- Paul Tavernier and Annick Linden (Belgium)
- Mirsada Hukic (Bosnia and Herzegovina)
- Anne Sofie Hammer (Denmark)
- Marja Isomursu (Finland)
- Olivier Mastain (France)
- Gudrun Wibbelt (Germany)
- Billinis Charalambos (Greece)
- Karoly Erdelyi (Hungary)
- Riccardo Orusa (Italy)
- Joseph Schon (Luxembourg)
- Andrea Groene (The Netherlands)
- Kjell Handeland (Norway)
- Patricia Santos (Portugal)
- Gabor Czirjak (Romania)
- Alexander Platonov (Russia)
- Sara Savic (Serbia)
- Gorazd Vengust (Slovenia)
- Christian Gortazar (Spain)
- Carl Hård (Sweden)
- Marie-Pierre Ryser (Switzerland)
- Ezgi Akdesir (Turkey)
- Paul Duff (U.K.)

Rev. sci. tech. Off. int. Epiz., 2011, **30** (3), 755-761

Establishing a European network for wildlife health surveillance



White: no data
Dark grey: comprehensive general wildlife health surveillance
Medium grey: partial general wildlife health surveillance (wide range of programmes but restricted in various ways)
Light grey: no general wildlife health surveillance, but some degree of targeted surveillance for a few specified diseases
Categories based on Leighton (13)

Fig. 1
Map of Europe depicting the level of wildlife health surveillance according to a self-evaluation of the participating countries (*n* = 25)

Goals of the EWDA network

- Improve exchange of information among wildlife health surveillance programs in Europe
- Develop standard operating procedures for diagnostic investigation
- Develop common criteria for diagnosis of wildlife disease
- Share specialist expertise
- Provide training opportunities for wildlife health surveillance

www.ewda.org

EWDA Website

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Diagnosis Cards

The aim of the **EWDA Diagnosis Cards** is to share and spread knowledge on appropriate diagnostic methods for diseases or disease agents in wildlife. More info here.

You can find the updated Diagnosis Cards below.

EWDA-DC_Avian_influenza.pdf View Download	Avian Influenza	193k	v. 1	14 Aug 2011 09:12	EWDA webpage
EWDA_DC_BT-EHD.pdf View Download	Bluetongue and Epizootic Haemorragic Disease	54k	v. 1	27 Sep 2012 00:30	EWDA webpage
EWDA_DC_Rabies.pdf View Download	Rabies	173k	v. 1	14 Nov 2012 10:00	EWDA webpage
EWDA_DC_TB.pdf View Download	Tuberculosis in wild mammals	185k	v. 2	16 Aug 2011 07:32	EWDA webpage
EWDA_DC_Trichinella.pdf View Download	Trichinellosis	139k	v. 2	24 Feb 2011 04:45	EWDA webpage

Network for wildlife health surveillance in Europe

EWDA Diagnosis Card

Trichinellosis

Author(s) (*corresponding author)

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Last update

18 February 2011

Etiology

Parasitic nematodes of the genus *Trichinella*. Eight species (*T. spiralis, T. nativa, T. britovi, T. murrelli, T. nelsoni, T. pseudospiralis, T. papuae* and *T. zimbabwensis*) and 4 genotypes (T6, T8, T9 and T12) are genetically and biologically delineated into 2 distinct clades characterized by the presence or not of an intramuscular collagen capsule (Table 1). Four species are currently encountered in European wildlife: *T. spiralis, T. britovi, T. pseudospiralis* and *T. nativa*.

Affected species (wildlife, domestic animals, humans)

Broad spectrum of potentially infected species involving domestic or wild mammals, birds, reptiles and humans, on all continents except Antarctica (no report or investigation carried out so far):

TABLE 1. Geographical distribution and host range of *Trichinella* species and genotypes (adapted from Gottstein et al., 2009).

Species and genotype	Geographical distribution	Typical host range
Encapsulated		
T. spiralis (T1)	Cosmopolitan	Domestic and sylvatic carnivores
T. nativa (T2)	Arctic and subarctic Europe, Asia, and North America	Sylvatic carnivores
Trichinella T6	Subarctic Canada and USA	Sylvatic carnivores
T. britovi (T3)	Temperate Europe and Asia, and North- Western Africa	Sylvatic carnivores and pigs
Trichinella T8	South Africa and Namibia	Sylvatic carnivores
T. murelli (T5)	USA and Southern Canada	Sylvatic carnivores
Trichinella T9	Japan	Sylvatic carnivores
T. nelsoni (T7)	Southern Africa	Sylvatic carnivores
Trichinella T12	Argentina	Cougars (*Felis concolor*)
Nonencapsulated		
T. pseudospiralis (T4)	Sporadically cosmopolitan	Sylvatic carnivores, birds of prey, pigs
T. papuae (T10)	Papua New Guinea, Thailand	Wild pigs, saltwater crocodiles (*Crocodylus porosus*)
T. zimbabwensis (T11)	Zimbabwe, Mozambique, Ethiopia, South Africa	Nile crocodiles (*Crocodylus niloticus*), monitor lizards (*Varanus spp.*)

Epidemiological characteristics and disease course

Transmission among animals: Ingestion of muscle tissue from an infected animal or consumption of infectious tissue from carrion of a homologous or heterologous species.
Domestic cycle: The focus is on a swine herd being fed, e.g., uncooked pork scraps, carrion, garbage (i.e., garbage-fed pigs), or on pigs allowed to feed on carcasses that are not promptly removed from the farm. Synantropic animals, particularly rodents, living near swine herd can contribute to the domestic cycle, which does not include all species/genotypes of *Trichinella*.
Sylvatic cycle: Transmission between wildlife hosts; includes all *Trichinella* species/genotypes (Table 1). Interaction between sylvatic and domestic cycle can occur when poor husbandry practices do not ensure strict separation between pigs and wildlife. *Humans*: Can be infected by eating raw or inadequately cooked meat which harbours infectious larvae.

Clinical signs

No clinical signs recognized in animals.
Clinical signs of acute trichinellosis in humans characterized by early (1) and later (2) phases: (1) nausea, diarrhoea, vomiting, fatigue, fever and abdominal discomfort; (2) muscle pains, headaches, fever, facial and eye swelling (oedema), aching joints, chills, cough and itchy skin. More severe cases are possible (including difficulties with coordinating movements, heart and breathing problems).

Gross lesions

No macroscopic lesions induced by *Trichinella* infection.

Histological lesions

Encapsulated or free larvae in the muscle.

Differential diagnosis

Other migrating nematode larvae recovered by digestion assay and/or leading to flu-like symptoms.

Criteria for diagnosis

Morphological criteria: Muscle larvae recovered by digestion assay are 1 mm long and 30 µm wide, contain stichosomes, and are not morphologically distinguishable to species or genotype.
Molecular identification: Multiplex PCR analysis generates DNA products that are unique in size for each species and genotype of *Trichinella* (Zarlenga et al., 2009).

Recommended diagnostic method(s) and preferred samples

Digestion assays are the only recommended procedures for the reliable detection of *Trichinella* larvae in meat. Different digestion assays are officially recognized in various countries for trade purposes. Assays other than those recommended by the International Commission on Trichinellosis (ICT) (documented standards in the EU, Canada or the USA) are not recommended. Trichinoscopy (examination of tiny pieces of meat by stereomicroscopy) is less sensitive and may be useful for rapid preliminary diagnosis. The EU reference method for detection of *Trichinella* larvae in meat is the magnetic stirrer method for pooled sample digestion (protocol in annex I, chapter 1 of the EC regulation 2075/2005; EEC, 2005). Analysis on fresh meat is recommended for human consumption. Freezing muscles prior to artificial digestion is possible for epidemiological studies on wild animals not designated for human consumption. The tongue and diaphragm of animals are main recommended sampling sites for the detection of all species/genotypes of *Trichinella* (Gajadhar et al., 2009).

TABLE 2. Predilection sites for *Trichinella* larvae in a few wild host species and size of samples to be examined.

Animal species	Predilection sites	Sample weight to be examined
Wild boar (*Sus scrofa*)	Forearm muscles, diaphragm, tongue	5 g
Fox (*Vulpes spp.*)	Diaphragm, forearm muscles, tongue	5 g at least
Bear (*Ursus spp.*)	Diaphragm, tongue, masseter muscle	10 g

Serology using the excretory/secretory antigens ELISA is recommended by ICT only for epidemiological surveys.

Laboratories that can be contacted for diagnostic support

French NRL for Parasites transmitted by Food. Animal Health Laboratory, Anses, France (www.anses.fr/index.htm)
EU Reference Laboratory for Parasites, Istituto Superiore de Sanita, Italy (www.iss.it/crlp/index.php)

Recommended literature

EEC. 2005. Regulation (EC) N° 2075/2005 of the European Parliament and of the Council of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. Official Journal of the European Community L 338: 60-82.

GOTTSTEIN, B., E. POZIO, AND K. NÖCKLER. 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clinical microbiology reviews 22: 127-145.

GAJADHAR, A. A.,E. POZIO, R. H. GAMBLE, K. NÖCKLER, C. MADDOX-HYTTEL, L.B. FORBES, I. VALLÉE, P. ROSSI, A. MARINCULIC, AND P. BOIREAU. 2009. *Trichinella* diagnostics and control: Mandatory and best practices for ensuring food safety. Veterinary Parasitology 159: 197-205.

OIE. 2008. Manual of diagnostic tests and vaccines for terrestrial animals, chapter 2.1.16 Trichinellosis. http://www.oie.int/esp/normes/mmanual/2008/pdf/2.01.16_TRICHINELLOSIS.pdf

ZARLENGA, D.S., CHUTE, M.B., MARTIN, A. AND KAPEL, C.M. 2009. . A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of *Trichinella*. International Journal of Parasitology 29:1859-1867.

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EWDA wildlife health network

120 of 128 topics ☆

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Welcome to the EWDA wildlife health network. This network consists of people who are EWDA members and/or are involved in or want to start up a wildlife disease surveillance scheme in Europe.

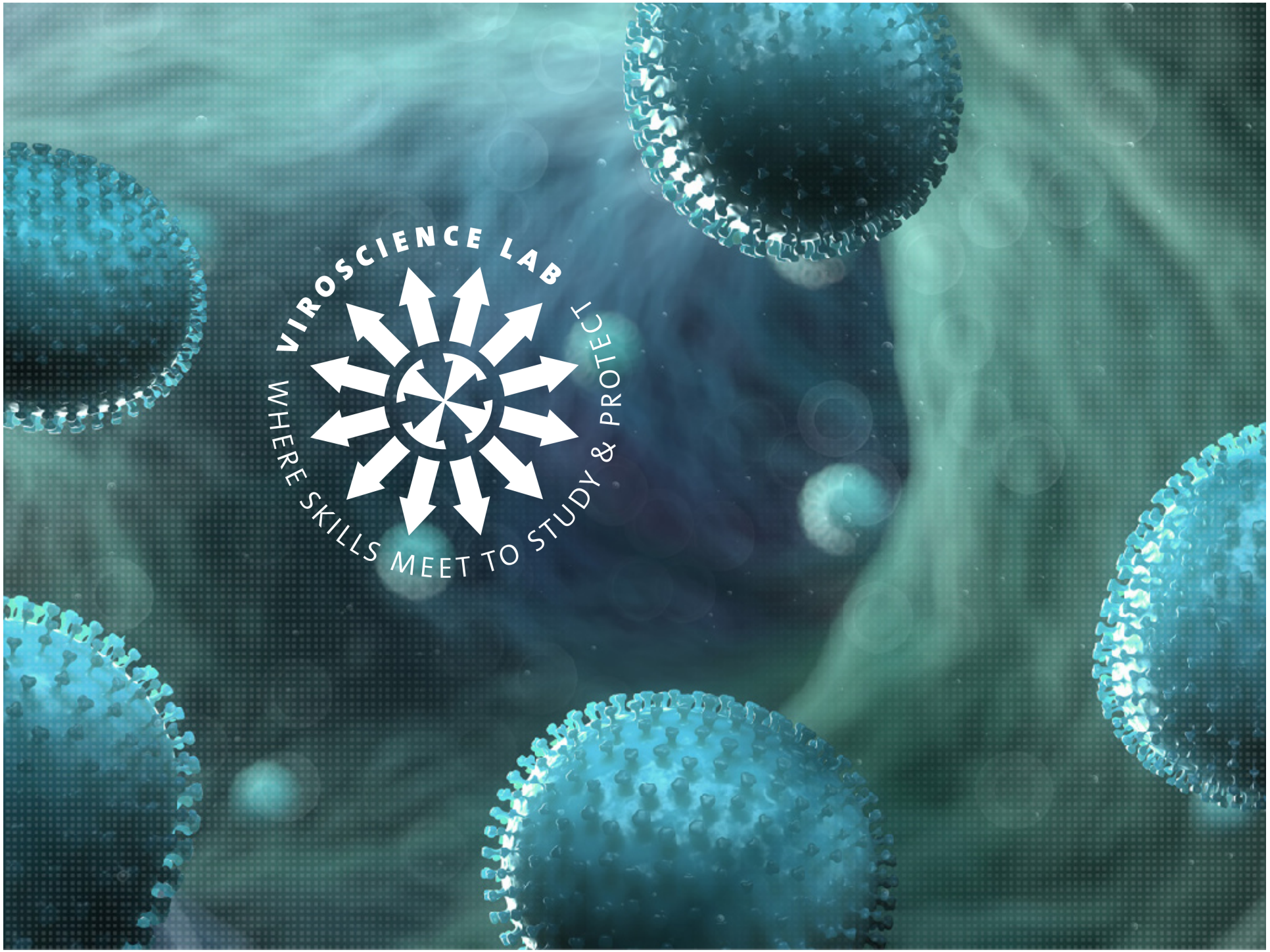
Edit welcome message Clear welcome message

- Fwd: PRO/AH/EDR> Snake fungal disease - USA (02): (TN)
  By Mainity Batista Linhares - 1 post - 2 views - updated Jun 17
- Fwd: PRO> Climate change, disease impact: Arctic spread
  By Mainity Batista Linhares - 1 post - 3 views - updated Jun 13
- MVetSci in Conservation Medicine
  By meredith - 1 post - 2 views - updated May 22
- Ecohealth training for veterinary students
  By Marc Artois - 2 posts - 2 views - updated May 21
- Fwd: Eurosurveillance, Volume 18, Issue 19, 09 May 2013
  By Marc Artois - 1 post - 4 views - updated May 13
- APHAEA meeting, 27-28 June 2013, Brescia, Italy
  By me - 1 post - 3 views - updated May 7

APHAEA: harmonised Approaches in monitoring wildlife Population Health, And Ecology and Abundance

- Overall aim: to establish a European wildlife disease network capable of providing reliable estimates of abundance of wildlife species and of pathogen distribution in these wildlife species

- Specific aims
  - To harmonize methods for estimating abundance of key wildlife host species
  - To harmonize methods for sample collection and diagnosis of key wildlife pathogens
  - To field-validate above methods on selected wildlife host-pathogen pairs
  - To develop a European wildlife disease network that uses above methods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