anses
French agency for food, environmental and occupational health & safety

Région Basse-Normandie

EU Reference Laboratory for equine diseases

OVERVIEW OF EU-RL CEM ACTIVITIES 2012-2015

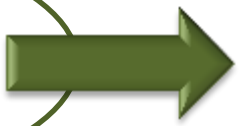
European CEM workshop – 1st October 2015

Speaker: Sandrine Petry

European Union Reference Laboratory for equine diseases
ANSES Dozulé Laboratory for equine diseases
Bacteriology and Parasitology Unit

Free from CEM is health and trade challenges for horse industry at National, European and International levels

National diagnostic,
National Reference &
EU Reference laboratories



Play a key role in CEM control

Need of an efficient network of laboratories with

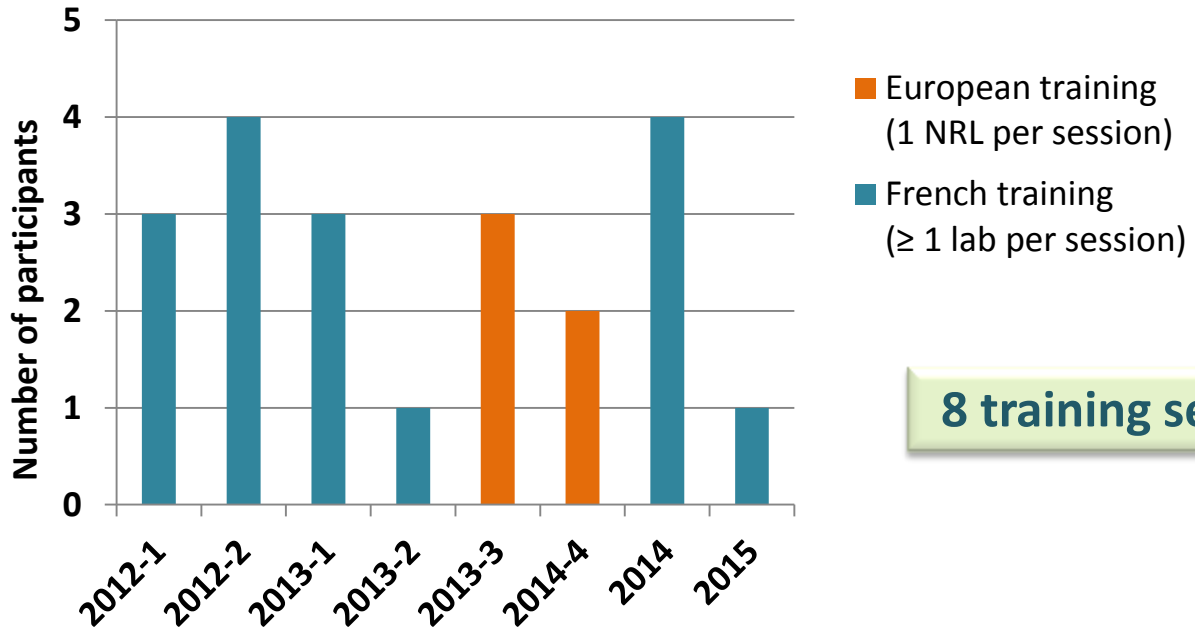
- Harmonized and powerful detection methods
- A technical support to confirm CEM cases and for molecular characterization of *T. equigenitalis* and *T. asinigenitalis* isolates
- A scientific support to better understand ecology of the CEM agent
- Constructive & friendly interactions despite the possible language barrier

THE EU-RL TOOLS AVAILABLE (CONTENTS)

- Training sessions
- Interlaboratory Proficiency Tests
- Providing strains & reagents
- Support for case confirmation & strain characterization
- Optimization of diagnostic tools
- Research on CEM

Training on CEM detection & diagnosis by culture, PCR and IF

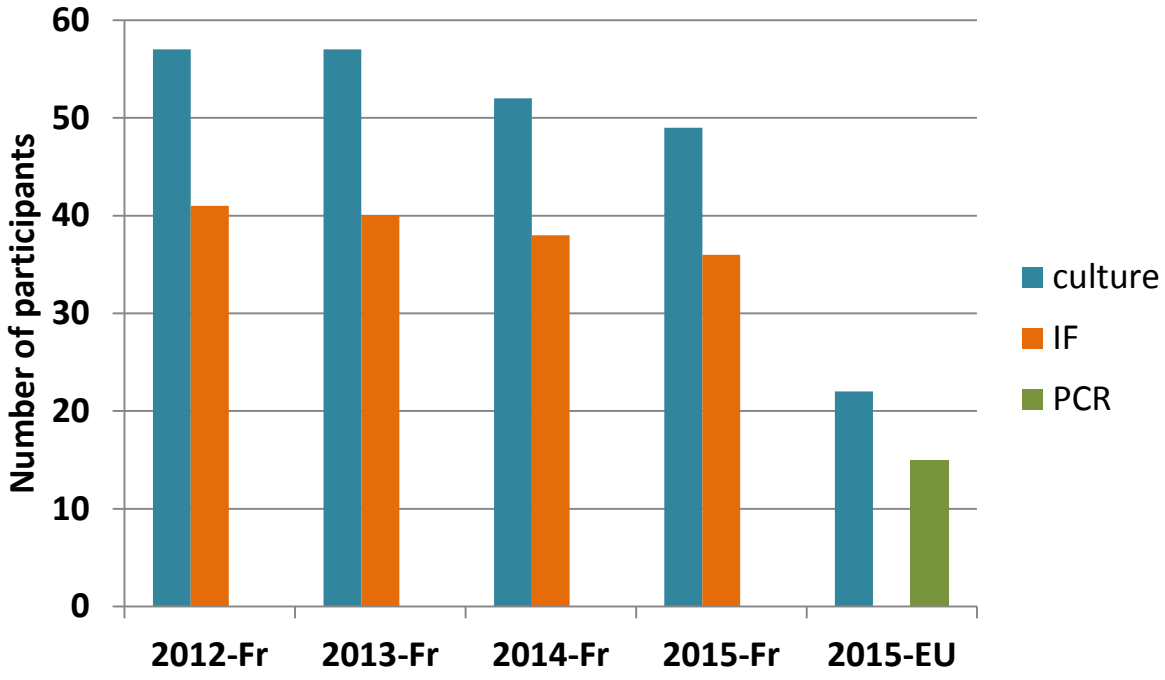
- Organized according to the requests
- Program can be adapted to the participants' needs
- In our lab or in the lab of the participant



8 training sessions in 4 years

Every year for France and every four years for Europe

- European PT are open to all methods in addition to the culture method
- Samples were artificially contaminated swabs

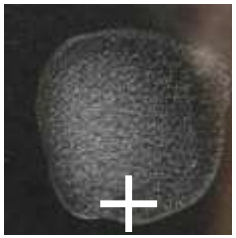


Characterized *Taylorella equigenitalis* and *Taylorella asinigenitalis* strains

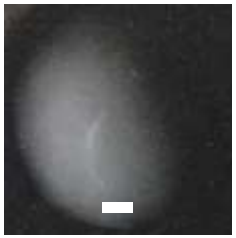
- NRLs → 6 *T. equigenitalis* and 11 *T. asinigenitalis*
- French labs → 25 strains

Polyclonal antibodies anti-*Taylorella equigenitalis* (produced on rabbits) for slide agglutination test*

- 1 NRL
- French laboratories performing the culture method (≈ 50)



T. equigenitalis

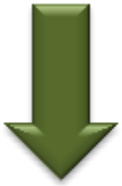


other bacteria and *T. asinigenitalis*

***Differential test during culture method alongside CO_2 dependency test**

Confirmation of CEM-positive cases

- No request from the European NRL network
- 8 French confirmation requests including 5 *T. asinigenitalis* suspicions
  - 2 CEM cases in February 2012 → free from CEM after these cases
  - 4 *T. asinigenitalis* cases out of the 5 suspicions



Project "Epidemiological study of *Taylorella* isolates circulating in Europe"

11:50 - 12:15 Molecular Epidemiology of *Taylorella* circulating in Europe (F. Duquesne, ANSES France)

Culture

- 5-15 µg/ml amphotericin B instead of 5 µg/ml (OIE chapter 2.5.2., 2012)
- 2 manufacturers provide the Timoney medium → BioMérieux & Oxoid

PCR

- 3 non-16S rRNA PCRs exist for detecting *T. equigenitalis*

✓ Anzai et al. (1999) semi-nested PCR
✓ Genekam Biotechnology AG nested PCR
✓ Genesig real-time PCR in DNA gyrase subunit B gene

In 2016, EU-RL will evaluate these PCRs and compare with the 16S rRNA PCRs

Immunofluorescence (IF)

- IDEXX provides a new formulation of monoclonal antibodies in its IF kit (Pourquier® IFI *Taylorella equigenitalis* Pool of mAb)

NEW FORMULATION OF MONOCLONAL ANTIBODIES

Specificity on strains
evaluated by EU-RL with 19 non-*T. equigenitalis* species

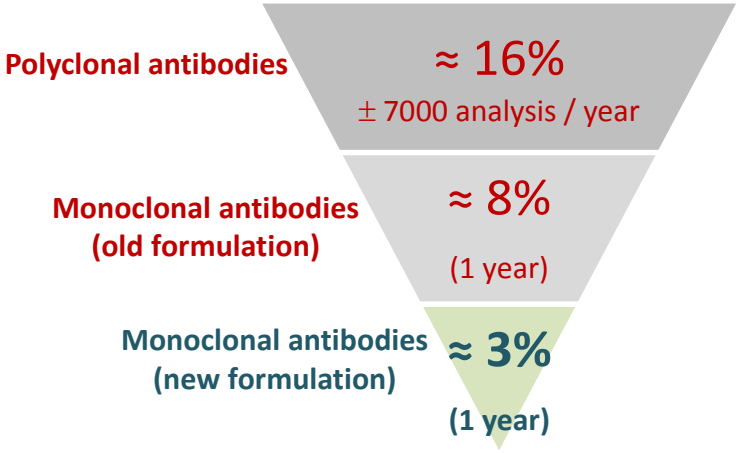
Specificity in field conditions
evaluated by a French diagnostic lab

Non-specific fluorescence or false positive results

→ *S. aureus, S. equi, S. equisimilis* & *S. zooepidemicus* with polyclonal and old monoclonal antibodies but not with monoclonal antibodies new formulation

→ all *T. asinigenitalis* tested (≈ 50) were negative using monoclonal & **polyclonal antibodies**

Modification of OIE Chapter 2.5.2



Sensitivity on strains
evaluated by EU-RL with 506 field isolates and 4 reference *T. equigenitalis*

100% positive
using monoclonal & polyclonal antibodies

Sensitivity and comparison with culture & PCR in field conditions
evaluated by CVI (The Netherlands)

14:20 -14:50 Comparison of PCR, IFT and culture as diagnostic methods for CEM (M. Engelsma, CVI The Netherlands)

1. **Improve the culture method with new media**
2. **Evaluate infectious models for future CEM studies**

12:15 - 12:40 Research of infectious model and attempt to improve the culture medium for taylorellae (L. Hébert, ANSES France)

Funders:

Collaborators:

EU Reference Laboratory for equine diseases

AGENDA OF THE DAY

09:00 - 09:40	**REGISTRATION**
09:40 - 10:00	Opening and participant introduction
10:00 - 10:15	Presentation of the on going CEM activities in Anses Dozulé laboratory for equine diseases (S. Petry, ANSES France)
10:15 - 10:30	Overview of CEM situation in individual countries since 2011 (S. Petry, ANSES France)
10:30 - 10:50	Epidemiology of CEM in Germany (F. Melzer, FLI Germany)
10:50 - 11:30	**COFFEE BREAK**
11:30 - 11:50	OIE Codes and Manual of Diagnostic Tests and Vaccines for Terrestrial Animals chapter on CEM (M. Dominguez, OIE)
11:50 - 12:15	Molecular Epidemiology of *Taylorella* circulating in Europe (F. Duquesne, ANSES France)
12:15 - 12:40	Research of infectious model and attempt to improve the culture medium for taylorellae (L. Hébert, ANSES France)
12:40 - 14:00	**LUNCH**
14:00 – 14:20	Pooling swabs for CEM PCR testing (N. Stamper, AHVLA UK)
14:20 -14:50	Comparison of PCR, IFT and culture as diagnostic methods for CEM (M. Engelsma, CVI The Netherlands)
15:00 - 15:15	Outcome of proficiency test about CEM diagnosis (S. Petry, ANSES France)
15:15 - 15:30	General discussion

Thank you for your attention