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Report of the 14<sup>th</sup> Workshop of  
the National Reference Laboratories  
for equine diseases  
(Subject: Equine Infectious Anemia and Dourine/Surra)

9-10 November 2022  
ANSES, Maisons-Alfort, France

## Introduction:

The programmes and all the presentations of these workshops are available on the website <https://eurl-equinediseases.anses.fr/>.

Nota bene: a preliminary inscription is necessary before having access to the private part of the website. The modality of this inscription is described in Annex 1.

## Workshop on EIA – November 9, 2022

The workshop was organised in hybrid mode: by visioconference and in-person. 65 participants coming from NRLs or a third country (Argentina, Tunisia, USA) were registered to attend this workshop (31 participants at Maisons-Alfort and 34 participants by visioconference). Ewa Camara was representing the European Commission. The agenda of the workshop is in Annex 2 of this report.

### **1. Opening and participant introduction (S. Zientara and G.Gonzalez, ANSES Maisons-Alfort - France)**

All the participants introduced themselves during the round table. Gaëlle Gonzalez, deputy head of the equine diseases EU-RL, presented the website (webmaster: G. Gonzalez) and the convivial event.

### **2. Animal Health Law: disease prevention and control rules applying to listed diseases of equine animals, in particular to equine infectious anaemia (E. Camara, European Commission SANTE/G2 )**

In introduction, Ewa Camara, reminded the listing and categorization of equine diseases and presented the Animal Health (AHL) legal framework. She gave an overview of the disease notification and reporting, surveillance, eradication programs, disease-free status (Part II of AHL), disease awareness, preparedness and control (Part III of AHL), the rules for movement between Member States of equine animals (Part IV of AHL) and the rules for entry into the European Union of equine animals (Part V of AHL). She focused on Equine infectious Anemia (EIA) that is categorized as a D and E disease. An EIA outbreak has to be notified in 24h through the Animal Disease Information System. Movement of horses between member states need to follow specific rules (no outbreak reported during 90 days or conduction of a diagnostic test (AGID or ELISA) if an outbreak was detected in the establishment in the last 12 months) accompanied by a certificate laid down in Regulation (EU) 2021/403.

**Please see:** Animal Health Law: disease prevention and control rules applying to listed diseases of equine animals, in particular to equine infectious anaemia (EIA); (Part: PT/workshops/Workshop presentations)

### **3. Update on Equine Infectious Anemia epidemiology data in Europe (J-C Valle-Casuso, ANSES, France)**

José-Carlos Vallé-Casuso presented the results of the EIA epidemiological survey 2018-2021 he sent to all NRLs. Sixteen of them replied to it. This survey included new questions as the age of the animals, the breed, the activity of positive-horses and a section dedicated to the diagnostic methods performed by each NRLS. He concluded that the measures applied by EU members are efficient as there is a decrease of the number of outbreaks declared by the most of the MS. The AGID technique is the one used mostly by the majority of the MS responding to the survey, only one country do not use this technique. ELISA assays are used by half of them. Two countries are integrated NGS activities in their EIA NRL activities.

## **Comments of the participants:**

One participant asked why only kits listed during the presentation (especially the one from IDEXX) are used by MS as there are other kits available in Argentina for example? J-C Valle-Casuso replied that different kits give different precipitation bands. To read those precipitation bands, you need an expert eye, which usually is adapted to a one kit. Here apparently most of the laboratories use IDEXX kit because their technicians found that this band is the easiest to read for them.

Stephan Zientara asked a question regarding nested PCR employed by Greece. This technique is performed as a complement to AGID or ELISA tests. Italy and Argentina are also employing immunoblotting as complement approach.

**Please see:** Update on Equine Infection Anemia epidemiology data in Europe by J-C Vallé-Casuso, ANSES (Part: PT/workshops/Workshop presentations)

### **4. Activity report on Equine infectious Anemia scientific news (J-C Valle-Casuso, ANSES, France)**

J-C Valle-Casuso gave an overview of relevant publications available on EIA published between 2018-2021. Over this period, 56 articles divided in 3 groups were published (viral cell cycle and cell – host/interactions, diagnostics techniques, and worldwide outbreaks). Member states are active mainly in the development of diagnostics methods.

#### **Comments of participants**

A participant asked if PCR may have a place in the future as diagnostic tools in case of EIA outbreak?

J-C Valle-Casuso emphasized that PCR is faster than the other methods and could reduce the time for the diagnostic, helping to avoid viral diffusion.

**Please see:** Activity report on Equine infectious Anemia scientific news by J-C Vallé-Casuso, ANSES (Part: PT/workshops/Workshop presentations).

### **5. Equine Infectious Anaemia Italian National Control Programme (Ida Ricci and Maria Teresa Scicluna, Istituto Zooprofilattico Sperimentale delle regioni Lazio e Toscana, Italy)**

An overview of the national surveillance plan put in place in Italy regarding EIA was given. Ten main labs constitute the network of the Italian reference laboratory for EIA. Positive samples detected by these labs are sent to the NRL confirm the diagnosis by performing ELISA and immunoblotting. There is an identification of low and high-risk areas for EIA over the country. They presented the Web-based geographic information system (WEBGIS) tool they developed for the control of EIA.

#### **Comments of participants**

A participant asked if they have an idea of the number of sanctuaries registered in Italy and if this is a feasible option for other member states. The presenter replied that each sanctuary is registered as an outbreak and that it is difficult to evaluate the number. This option can be an “in-between” solution and can help ensuring biosecurity measures, vets being in constant vigilance.

Dr Fussel highlighted that distance should be at least 200 meters to keep contaminated horses from neighbor “free” establishments.

**Please see:** Equine Infectious Anaemia Italian National Control Programme by Ida Ricci and Maria Teresa Scicluna, Istituto Zooprofilattico Sperimentale delle regioni Lazio e Toscana, Italy. (Part: PT/workshops/Workshop presentations).

## **6. Equine Infectious Anaemia Whole Genome sequencing protocol (J-C Valle-Casuso, ANSES, France)**

J-C Vallé Casuso presented the first step of the development of an innovative tool for molecular diagnosis (NGS-based / primer-free method). Thanks to it, 14 new complete sequences of EIAV were obtained. There are 38 strains to be sequenced following the same pipeline.

### **Comments of participants**

A participant asked how to deal with strains obtained from horse samples that may contract the disease elsewhere. Dr Valle-Casuso answered that generating a phylogenetic tree would be the best to make sure about the origin of the strain, but for that we need to advance in the identification of EIAV strains using modern sequencing techniques.

S. Zientara asked why there are conserved regions identified for HIV and not for EIAV. Dr Valle-Casuso answered that EIAV is not characterized enough to do that for the moment. This information, could be only obtained once from a hundred to thousands of strains of EIAV will be sequenced

**Please see:** Equine Infectious Anaemia Whole Genome sequencing protocol by J-C Vallé-Casuso, ANSES (Part: PT/workshops/Workshop presentations).

## **7. PCR Commercial kit tested by the EURL Team (J-C Vallé-Casuso, ANSES, France)**

There are 4 PCR commercial kits available to detect EIAV. The EURL team tested two kits on 42 EIAV-positive samples. The first one detected nothing, and the second one detected half of the positives but is no more available since this summer. The EURL team tested 6 non-commercialized molecular biology protocols for EIAV detection in a panel of 13 EIAV positive samples, and the best of them identified 8 positives (61%). Taking advantage from the new EIAV sequences completed in the EURL lab and those with more than 85% of the sequence complete, a consensus sequence was built, and 25 primer sets were designed. The preliminary results from 2 couple of primers of this set of 25, are promising if we use those in the future as a multiplex protocol.

### **Comments of participants**

A participant asked if the non-commercialized PCR and the whole genome sequencing tools could be used from live animals' samples. This need to be tested after a concentration of targeted cells infected by EIAV as macrophages.

S.Zientara questioned the sensitivity of HIV PCR. Do we miss virus? J-C Valle-Casuso is confident in the identification of all positive cases as today, our techniques allow us to identify the HIV by extract RNA/DNA from one single cell.

**Please see:** PCR Commercial kit tested by the EURL Team by J-C Valle-Casuso, ANSES (Part: PT/workshops/Workshop presentations).

## **8. State of the art of antiviral molecules available against retroviruses (PhD student, Cécile Schmmich, ANSES, France)**

An overview of antiretroviral molecules available against HIV and their action mode during the replication cycle of the virus was explained by C.Schmmich. The question is now to use these molecules against EIAV. The first part of her PhD project is to test the antiviral properties of 17 of them on EIAV replication in equine dermal fibroblasts and PBMCs. She presented preliminary results to the member states.

### **Comments of participants**

A participant asked if this could be an option in order to avoid killing EIAV positive animals. E. Camara replied that we need to get the authorization from the EU and the stakeholders. J-C Valle-Casuso added that this is the beginning of a research project to get tools to block viral replication, but we are far from proposing an antiviral treatment against the EIAV.

**Please see:** State of the art of antiviral molecules available against retroviruses by C Schmmich, ANSES, France (Part: PT/workshops/Workshop presentations).

### **9. Equine Infectious Anemia surveillance in the USA (M.K Torchetti, USDA, Iowa)**

Dr Torchetti presented the surveillance system implemented in the USA and the epidemiological data regarding EIA. A decrease in the number of outbreaks reported in 2022 was noticed compared to 2021.

#### **Comments of participants**

A participant asked if it is possible to use USDA licensed test to allow import of horses to the USA from South America. Another question was about

**Please see:** Equine Infectious Anemia surveillance in the USA by M.K Torchetti, USDA, Iowa (Part: PT/workshops/Workshop presentations).

### **10. Equine Infectious Anemia surveillance program in South America (A. Vissani, Argentina)**

Dr Vissani presented the surveillance system implemented in Argentina and the epidemiological data regarding EIA. A decrease in the number of outbreaks reported in 2022 was noticed compared to 2021. EIA is endemic in the North of the country. Research projects focused on the development of diagnostic tests using a recombinant p26 antigen and on the use of isothermal RT-PCR to detect EIAV. This presentation emphasizes the importance of sustaining EIA surveillance in the area considered as “low prevalence” in Argentina, as this area is where the most valuable horses are bred in Argentina

#### **Comments of participants**

A participant asked about the sensitivity of the isolated isothermal PCR and the immunoblotting compared to AGID and ELISA tests. Would it be easier to detect earlier positive horses using those tests?

A. Vissani replied that sera of EIA positive horses diagnosed by AGID or ELISA were used to validate the isothermal PCR test. Another comment was about the reagents used to do the immunoblotting and their origin. Dr Vissani replied that they generate their own reagents.

**Please see:** Equine Infectious Anemia surveillance program in South America by A. Vissani (Argentina) (Part: PT/workshops/Workshop presentations).

### **Outcome of Proficiency test on EIA serology AGID and ELISA (D. Froger - Anses)**

Delphine Froger presented the outcome of the AGID and ELISA proficiency tests, which included 24 and 17 participants laboratories respectively.

EIA AGID ILPT conclusion:

Given the results obtained by the 24 participant laboratories, 18 out of them obtained satisfactory results. PhEED UNIT will guide the participant laboratories that obtained unsatisfactory results in implementing corrective actions and to monitor their 14<sup>th</sup> workshops of NRLs for equine diseases, 9-10 November 2022

effectiveness. Moreover, a training session will be proposed to them. Overall, the results obtained by the European NRL network are very good according to the specificity (99.3%) and sensitivity (97.7%) of this ILPT.

EIA ELISA ILPT conclusion:

Given the results obtained by the 17 participant laboratories, 16 out of them obtained satisfactory results. The participant laboratory that obtained three unsatisfactory results obtained 100% of satisfactory results after implementing corrective actions and monitoring their effectiveness by testing again the three samples they failed to detect well. Overall, the results obtained by the European NRL network are very good in this IL-PT according to the specificity (100%) and sensitivity (99.3%).

**Please see: Please see:** 1/ Outcome of Proficiency test on EIA serology (AGID) by D. Froger (part workshop\_ private access) and 2/ Outcome of Proficiency test on on EIA serology (E) by D. Froger (Part: PT/workshops/Proficiency test).

The workshop was organised in hybrid mode: by visioconference and in-person. 55 participants coming from NRLs, or a third country (Argentina, Iran, USA) as well as our previous desk-officer d'Alf-Eckbert Füssel were registered to attend this workshop (28 participants at Maisons-Alfort and 27 participants by visioconference). Ewa Camara was representing the European Commission. The agenda of the workshop is in Annex 2 of this report.

### **1. Opening and participant introduction (S. Zientara and G. Gonzalez, ANSES Maisons-Alfort - France)**

All the participants introduced themselves during the round table. Gaëlle Gonzalez, deputy head of the equine diseases EU-RL, presented the website (webmaster: G. Gonzalez).

### **2. Animal Health Law: disease prevention and control rules applying to listed diseases of equine animals, in particular to Dourine and Surra (E. Camara, European Commission SANTE/G2 )**

In introduction, Ewa Camara, reminded the listing and categorization of equine diseases and presented the Animal Health (AHL) legal framework. She gave an overview of the disease notification and reporting, surveillance, eradication programs, disease-free status (Part II of AHL), disease awareness, preparedness and control (Part III of AHL), the rules for movement between Member States of equine animals (Part IV of AHL) and the rules for entry into the European Union of equine animals (Part V of AHL). She focused on Dourine and Surra that are categorized as a D and E diseases. Movement of horses between member states need to follow specific rules (no Surra outbreak reported during 30 days in the establishment and no Dourine cases reported during 6 months). Additional conditions are applicable if a case was reported in the last 2 years in the establishment (CFT for Dourine, ELISA or CATT/T.evansi for Surra).

#### **Comments of participants**

A participant asked how requirement about Surra and Dourine is phrased in the EU legislation. A country without reported cases for two years even if the disease is endemic (EU need to be informed) can export horses. The EU commission take into consideration the epidemiological situation of the country regarding importation of horses.

**Please see:** Animal Health Law: disease prevention and control rules applying to listed diseases of equine animals, in particular to Dourine and Surra by E. Camara, EU commission (Part: PT/workshops/Workshop presentations).

### **3. Activity report on Dourine and Surra: scientific news (Laurent Hébert, ANSES, France)**

Laurent Hébert gave an overview of relevant publications available on Dourine and Surra, the main reference and research missions of the EURL Team, control reagents, positive sera available for MS. He presented the WOAHP definition for Surra and Dourine cases in equids and the diagnosis challenges of Dourine, Surra and Nagana.

#### **Comments of participants**

A participant asked which tests and methods are employed by the EURL team to eliminate a false positive Dourine sample. L. Hébert replied that they have to wait for the serology (CFT) to become negative. The USDA test the animal every two weeks. They estimated that if the horse is infected the titer will not decrease over time.

Another question was about other pathogens that can cross react during the CFT diagnostic test. L. Hébert hypothesized that vaccination against Rhinopneumonia can induce false positive for Dourine. Another point on CATT/T.evansi and dog false positive results was discussed. Nick Van Reet, that could not assist to the meeting, was the best person to answer this point.

Another participant asked a question about the phylogenetic trees generated for Dourine to know if the Italian strains from the 2011 Dourine outbreak had been characterised and if positive cases developed clinical symptoms. L. Hébert replied that the phylogenetic classification of the strains from the Italian outbreak had not been fully described but results obtained by Achim Schnauffer (Edinburgh University) suggested that the strains were related to the clade *T. evansi* type B strain. L. Hébert also confirmed that it is expected that infected horses develop clinical symptoms.

**Please see:** Activity report on Dourine and Surra: scientific news by L. Hébert, ANSES, France (Part: PT/workshops/Workshop presentations).

#### **4. Equine trypanosomes in Iran (1876-2022): a literature review (A. Sazmand, Bu-Ali Sina University, Iran)**

Dr Sazmand recorded a video for his presentation. He presented an overview of Dourine and Surra surveillance program implemented in Iran.

The video could be sent to participant on demand.

#### **5. Dourine (*T. equiperdum*) and Surra (*T. evansi*) serologic surveillance in Argentina during 2019, 2020, 2021 and 2022 (T., Becù, Clinica Equina SRL, Argentina)**

T. Becù gave an overview of the epidemiological situation of Dourine and Surra in Argentina over the last four years. Dourine is “an exotic” disease in Argentina whereas Surra is endemic in the North and North West of the country.

**Please see:** Dourine (*T. equiperdum*) and Surra (*T. evansi*) serologic surveillance in Argentina during 2019, 2020, 2021 and 2022 (T., Becù, Clinica Equina SRL, Argentina) (Part: PT/workshops/Workshop presentations).

#### **6. Development of new tests to increase the performance of Dourine's diagnosis (M. Tittarelli, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Italy).**

M. Tittarelli gave an overview of the epidemiological situation of Dourine and Surra in Italy. She focused on the fact that there is an urgent need to identify unique proteins of *T. equiperdum* that could be used to improve serological diagnosis of Dourine. The rabbit experimental model was proposed to replace horse experimental infection as it gave identical results by CFT and immunoblotting.

**Please see:** Development of new tests to increase the performance of Dourine's diagnosis by M. Tittarelli, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Italy (Part: PT/workshops/Workshop presentations).

#### **7. Development of two alternatives for trypanosomosis diagnosis (Laurent Hébert, ANSES, France)**

Dr Hébert presented the development of a micro-sphere based immunoassay for the serological diagnosis of equine trypanosomosis. He identified an antigen suitable for Luminex: GM6. He also presented the EURL work on diagnosis based on the 7SL-sRNA detection, which has the specificity of molecular detection while being independent of the direct presence of the parasite.

**Please see:** Development of two alternatives for trypanosomosis diagnosis by L. Hébert, ANSES, France (Part: PT/workshops/Workshop presentations).



## 8. *In-vitro* production of *Trypanosoma equiperdum* antigen for CFT – time consuming, expensive, but possible (Gereon Schares, Friedrich-Loeffler-Institut, Germany)

Dr Schares presented the *in vitro* production of *T. equiperdum* antigen and its evaluation for use in serodiagnosis of Dourine. The FLI-CFT based on *in-vitro* generated antigen passed the PT test offered by the EURL team in 2022.

**Please see:** *In-vitro* production of *Trypanosoma equiperdum* antigen for CFT – time consuming, expensive, but possible by Gereon Schares, Friedrich-Loeffler-Institut, Germany (Part: PT/workshops/Workshop presentations).

## 9. Outcome of proficiency test about serology of Dourine (L. Hébert, ANSES, France)

L. Hébert gave the outcome of the PT test about serological diagnosis of Dourine and Surra. 81% and 67% of the laboratories returned satisfactory results regarding respectively Dourine CFT and CATT/T.evansi.

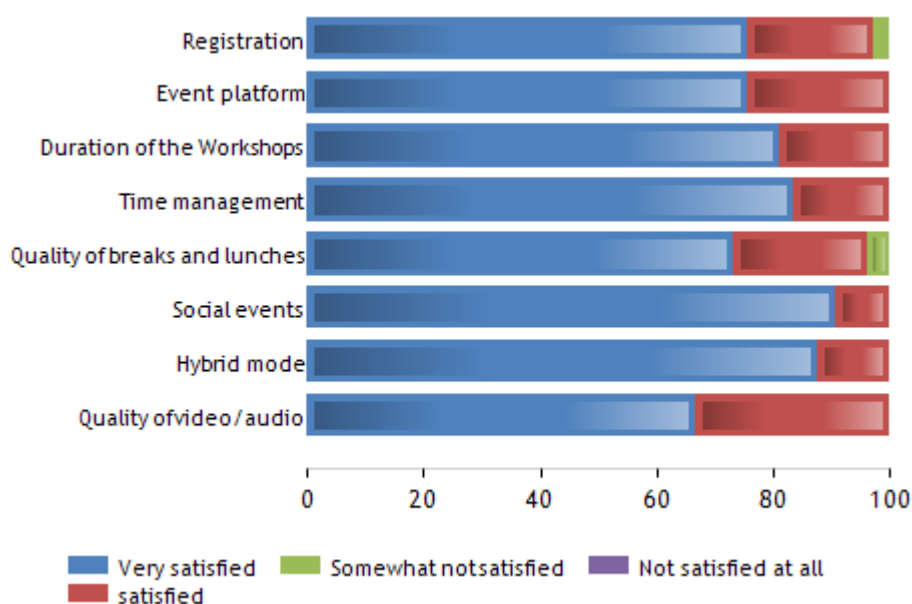
**Please see** Outcome of proficiency test about serology of Dourine by L. Hébert, ANSES, France (Part: PT/workshops/Proficiency test).

### EU RL workshop 2021 - Analysis of workshops on EIA and Dourine/Surra

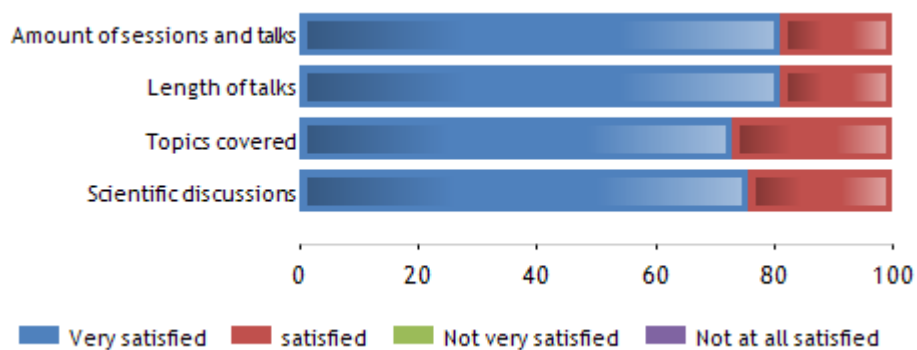
A survey and global evaluation of the hybrid mode EIA and Dourine/Surra workshops was sent on November 26 to know the feedbacks of NRLs. 38 answers for these workshops were received from participant. 48.6% of the participants that replied to the survey were in-person in Maisons-Alfort and 51.4% were by visioconference.

The evaluation of each item followed this graduation scale: **very satisfied, satisfied, not very satisfied, not at all satisfied.**

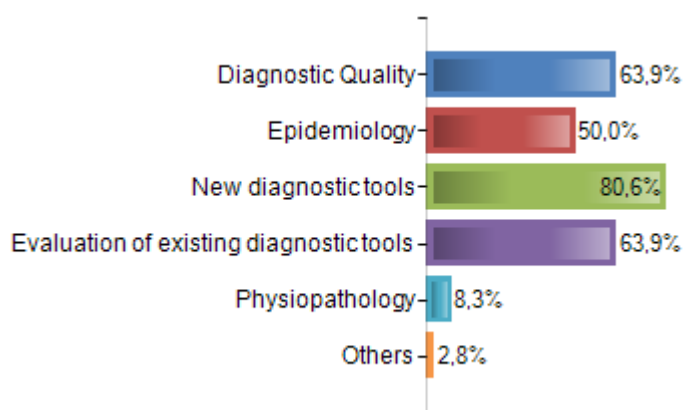
#### Content: Workshops organisation



### Content: EIA and Dourine/Surra Workshops presentations

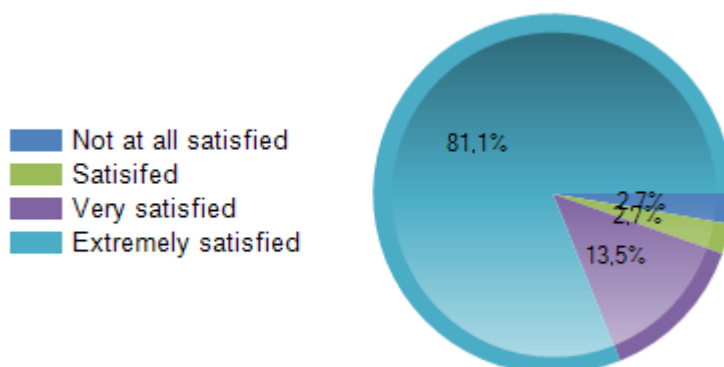


### Content: topics participants would you like to see more of at the 2023 Workshops



Comments: one participant mentioned virus- host interactions and evolution of virus strains in the arms race

### Content: Overall satisfaction



Comments: one participant mentioned to be not at all satisfied but it seems that it is a mistake as it clicked very satisfied until this point.

- Have you any other comments or proposal concerning the organisation of the workshop?

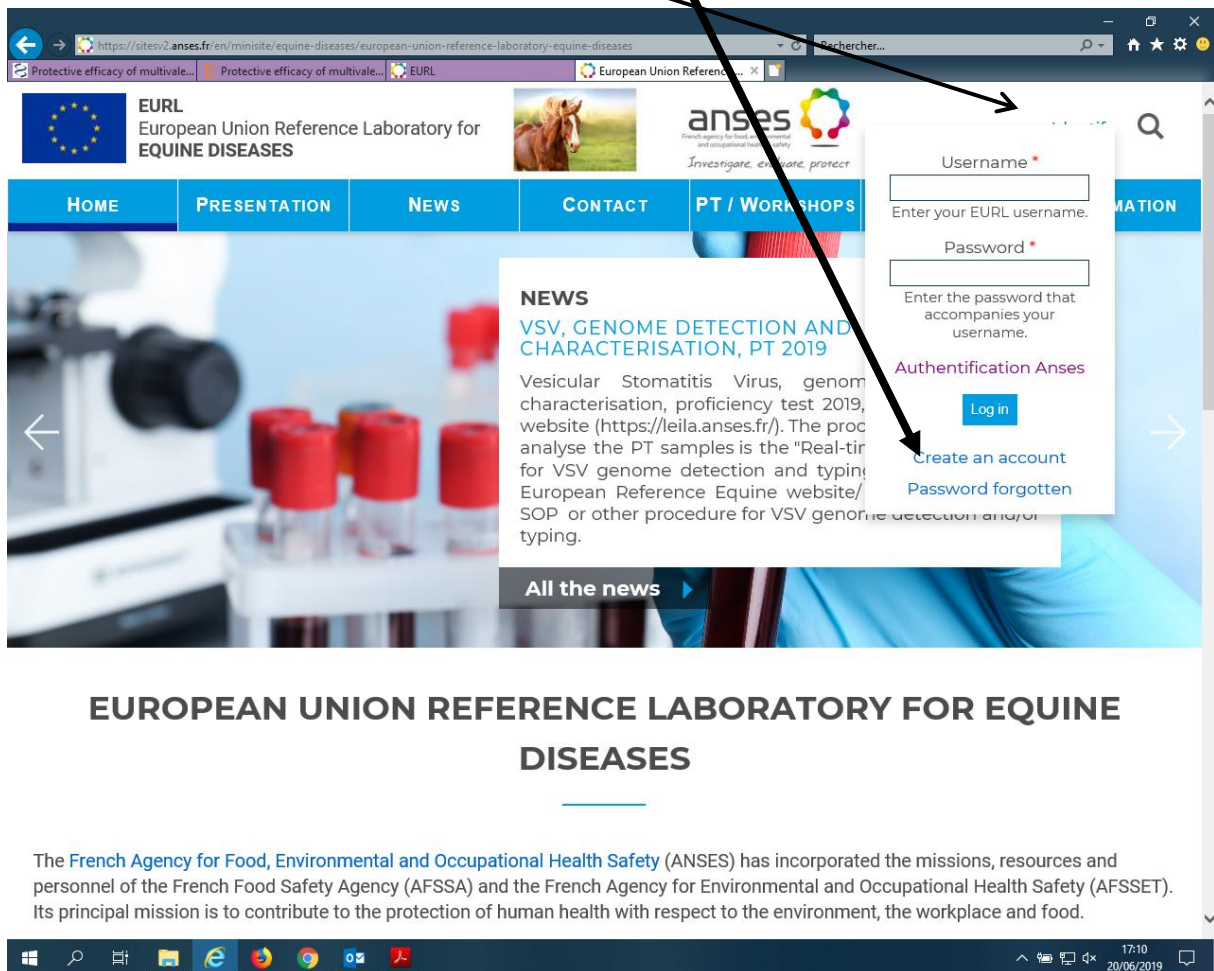
Participant's comments		EU RL response
1	I really appreciated the possibility to participate remotely, thank you!	We will try to set up an automatic reply system via email after each registration.
2	I personally believe that it would be more convenient for all of us if the expenses are covered in advance for all the participants as it is the case for other workshops organized by other EURLs (indicatively I mention colleagues from Montpellier, EURL for PPR) taking into consideration the costly and time-consuming existing procedure.	We will propose this to our financial support team and will come back to you.
3	Pleasant and well organized event	It was a pleasure to organize these workshops in Maisons-Alfort and by visioconference after the COVID19 pandemic

To create an account

Home and presentation are in open access; the others links (news, contacts ....) are in private access.

The URL is: <https://eurl-equinediseases.anses.fr>

To go to the private access you have to select “identify” and “create an account”. Choose Microsoft Edge, chrome or Firefox.



You can choose (and remember!!) your username and password

The validation Key is : EQUINE2014

When you have submitted your registration you will receive a confirmation of your submission by email.

After validation of your inscription by the webmaster, you will receive by email a confirmation of your inscription and you'll be able to connect to the private access.

# Workshops of the European Reference Laboratories for Equine Infectious Anemia and Dourine-Surra

## Agenda

9<sup>th</sup> and 10<sup>th</sup> November 2022

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Maisons-Alfort, France



## Workshop of the European Reference Laboratories for Equine Infectious Anemia

**Wednesday, November 09, 2022**

- 9:00** Registration – Welcome Coffee
- 9:30** Opening Session / Website actualities / Social event (**S. Zientara, G. Gonzalez, ANSES, France**)
- 9:50** Animal Health Law: disease prevention and control rules applying to listed diseases of equine animals, in particular to equine infectious anemia (**E. Camara - EC**)
- 10:20** Update on Equine infectious anemia epidemiology data in Europe (**J-C. Valle-Casuso, ANSES, France**)
- 10:40** Activity report on Equine infectious anemia: scientific news (**J-C. Valle-Casuso, ANSES, France**)

**11:00 Coffee Break**

- 11:30** Participant presentations (**Dr. Scicluna Maria Teresa and I. Ricci, Istituto Zooprofilattico Sperimentale delle regioni Lazio e Toscana, Italy**)
- 12:00** EIA whole-genome Sequencing Protocol (**J.C Valle-Casuso, ANSES, France**)
- 12:15** PCR Commercial kit tested by the EURL team (**J.C Valle-Casuso, ANSES, France**)
- 12:30** State of the art of Antiviral molecules available against retroviruses (**PhD student Cécile Schmmich, ANSES, France**)

**13:00 Lunch Break**

- 14:15** EIA surveillance program in the USA (**M.K Torchetti, USDA, Iowa**)
- 14:30** EIA surveillance program in South America (**Aldana Vissani, Instituto de Virología, CICVyA. INTA, Argentina**)
- 15:00** Outcome of proficiency test on EIA serology (AGID) (**D. Gaudaire, ANSES France**)
- 15:45** Outcome of proficiency test on EIA serology (ELISA) (**D. Gaudaire, ANSES France**)
- 16:10** Group Photo
- 18:00** Meeting point at the City Island followed by the dinner at the Chai33 restaurant.

## Workshop of the European Reference Laboratories for Dourine/Surra

Thursday, November 10, 2022

- 9:00** Registration – Welcome Coffee
- 9:30** Opening Session / Website actualities / General information (**S. Zientara, G. Gonzalez, ANSES, France**)
- 9:50** Animal Health Law: disease prevention and control rules applying to listed diseases of equine animals, in particular to Dourine/Surra (**E. Camara - EC**)
- 10:15** Activity report on Dourine and Surra: scientific news (**L. Hébert, ANSES, France**)

**11:00 Coffee Break**

- 11:30** Equine trypanosomoses in Iran (1876–2022): a literature review (**A. Sazmand, Bu-Ali Sina University, Iran**).
- 12:00** Dourine (*T. equiperdum*) and Surra (*T. evansi*) serologic surveillance in Argentina during 2019, 2020, 2021 and 2022 (**T. Becú, Clinica Equina SRL, Argentina**).
- 12:20** Development of new tests to increase the performance of Dourine's diagnosis (**M. Tittarelli, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Italy**).
- 12:35** Development of two alternatives for trypanosomosis diagnosis (**L. Hébert, ANSES, France**).

**12:50 Lunch Break**

- 14:00** *In-vitro* production of *Trypanosoma equiperdum* antigen for CFT – time consuming, expensive, but possible (**Gereon Schares, Friedrich-Loeffler-Institut, Germany**).
- 14:15** Outcome of proficiency test about serology of Dourine (**L. Hébert, ANSES, France**).
- 14:35** Closure of the Workshop – Group Photo.