

## **Summary of EU Reference Laboratories meeting on African Swine Fever (ASF) and Classical Swine Fever (CSF)**

The meetings were organized in Brussels by the European Commission.

Locations: JACQUES DELORS BUILDING and VAN MAERLANT BUILDING

Dates: 3 – 4 June 2013. The first day of the meeting was on ASF the second one on CSF

Attendances: 52 delegates from 29 countries: EU Member States and Third countries.

Delegates from Serbia and Russian Federation attended the meetings, Russia only the first day (ASF).

### **During the ASF meeting (June 3) the following topics were presented and discussed:**

- ASF update: gaps and needs to improve laboratory diagnostics
- Country reports situation: Italy
- EURL: activities and research concerning sample collection and diagnosis during 2012-2013
- EURL: Results of the Inter-laboratory Comparison Test 2012-13
- Laboratory Contingency Plans
- Biological and molecular characterization of ASFV isolated in Ukraine, 2012
- Lectures by participants on Epidemiology and Diagnosis
- ASF Global Research Alliance: Research perspectives in the future

### **Conclusions ASF**

1. ASF is still present in Sardinia with an epidemic season in 2012-2013 highlighting new infected areas in the north part of the island. Pig production in small family herds, illegal movement, and swill feeding of pigs, are critical points for the control of the disease.
2. ASF is established in the South-West areas of Russian Federation (RF), affecting both domestic pigs and wild boars with on-going northwards spread observed during 2012.
3. The presence of ASF specific antibodies in both domestic pigs and wild boar in Russia territories has been demonstrated through a twinning program between the OIE (UCM, Madrid) and NRIVVaM (Pokrov, Russia), indicating the possibility of an endemicity pattern.

4. For the first time, Ukraine declared an outbreak in July 2012 which was produced by a highly virulent ASFV isolate belonging to the same genotype II circulating in Russia and the Caucasus region. The data demonstrated the gradual spread of the disease increasing the risk for ASF introduction in the EU countries.
5. All NRLs belonging to the European Union have participated in the X-ILCT for ASF, performing the serological and virological ASF diagnosis in both serum and tissue samples. However, confirmatory antibody detection test is not performing by all NRLs.
6. The results obtained in the X ILCT for ASF have been satisfactory and the 88% (22/25) of the NRLs belonging to the EU provided a correct final ASF diagnosis in all samples.
7. New PCR techniques such as UPL PCR (Fernández-Pinero *et al.*, 2013) and Taqman Real time (Tignon *et al.*, 2011) incorporated at NRLs have proved increased sensitivity in the detection of carrier animals.
8. The interlaboratory validation of the Indirect Immunoperoxidase technique (IPT) performed in the X-ILCT has showed this technique as a validated alternative confirmatory serological test.
9. Whole blood collected on filter paper (DB samples) has been shown to be adequate sources of antibodies for laboratory testing using the “ID Screen® ASF Indirect ELISA KIT IDVET [CODE ASFBI]” and IPT aiming detection of antibodies to ASF.
10. Non-invasive samples such as oral fluid have been demonstrated as a potential alternative sampling method for antibody detection.

### **Recommendations ASF**

1. The current epidemiological situation in Sardinia, and the progression towards a pattern of endemicity in Russian Federation, point out the need for better characterization of the endemic state in the affected areas.
2. Improved knowledge of the virus evolution in affected European countries, and the role of survivors/carriers in the transmission of the disease should be better investigated.

3. It is important to maintain an ongoing assessment of the diagnostic techniques that allow the detection of endemic situations with appropriate sensitivity levels.
4. The epidemiological role of the wild boar in affected areas should be better assessed.
5. It is recommended at the NRLs to confirm the positive and doubt results obtained by the ELISA screening tests using serological confirmatory tests such as IB, IPT or IFI.
6. An accurate evaluation of the results of the diagnostic tests must be carried out, taking into account all the clinical, the epidemiological findings, and both serological and virological results.
7. To manage an emergency disease situation it would be desirable to have knowledge about the commercial ELISA kits available in stock. NRLs should consider having an alternative diagnostic test ready to use.

**During the CSF meeting (June 4) the following topics were presented and discussed:**

- CSF situation 2012/13 in Latvia
- CSF situation 2012/13 in Bulgaria
- CSF situation 2012/13 in Romania
- Results of the Inter-laboratory Comparison Test 2012/13 and activities performed
- The new CSF database at the EU and OIE Reference Laboratory
- Experience with the use of a genetic DIVA concept after oral immunization of wild boar
- The evolution of the CSF sero-prevalence in the Northern Vosges, France
- Survival of CSFV in porcine tissues

**Conclusions CSF**

1. In 2012, 56 laboratories from 47 countries participated in the CSF ILCT 2012. Results indicate that EU laboratories are well prepared to diagnose CSF. Laboratories who wish to improve their results will perform corrective actions that will be followed by a second round of testing. The EURL for CSF will contact the respective laboratories.

2. Apart from the limited outbreaks in wild boar and three backyard holdings in Latvia in 2012/2013 no new outbreaks were identified within the Member States.
3. In 2012 testing of serum samples from domestic and Eastern Balkan pigs in Bulgaria revealed a low number of positive results in the CSFV antibody ELISA. Further investigations of samples from these holdings resulted in isolation of BVDV and demonstrated the absence of CSFV.
4. The new CSF database at the EU and OIE Reference Laboratory provides easy-to-use-tools for genetic typing of new CSFV isolates and detailed phylogenetic analyses of CSFV sequences including 5'NTR, E2 fragment, and entire E2 encoding sequences. Full-length E2 sequences provide valuable data to characterize genetic relationship of CSFV isolates in more detail.
5. A genetic DIVA real-time RT-PCR assay for differentiation of C-strain vaccine virus from CSFV field viruses has been established and can be used after oral immunization of wild boar.
6. Vaccination of wild boar in France stopped in June 2010. In some formerly vaccinated, small, limited areas low sero-prevalence, even in the younger animals, is still detected.
7. Thermal inactivation curves for CSFV in porcine tissues have been determined by virus isolation. The data can inform estimates of the how long CSFV remains viable at different temperature in porcine tissues.

### **Recommendations CSF**

1. It was recommended to send the BVDV isolates from Bulgaria to EURL for CSF for further characterization.
2. For the next ILCT NRLs are encouraged to provide Ct values when submitting their real-time RT-PCR results.
3. For antibody ELISA testing it could be helpful to heat inactivate doubtful or questionable serum samples (30-60 minutes at 56 °C).
4. For next ILCT NRLs are encouraged to include sequence analysis and genetic typing on PCR positive samples. The new virus database of the EURL and GenBank (blast) are recommendable tools.
5. To achieve a higher resolution of phylogenetic analyses of CSFV sequences it is recommended to use full-length E2 sequences.
6. For genetic DIVA real-time RT-PCR it is recommended to optimize sensitivity and specificity by modifications regarding extraction methods, cycling parameters and pre-treatment of samples.
7. Further investigations should explain the origin of seropositivity still detected in the age class of piglets in wild boar in France.