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Rescue of the CSFV Koslov strain from a cloned cDNA

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"Workshop on Laboratory Diagnosis of African and Classical Swine Fever (ASF and CSF)"

June 2nd and 3rd 2014

Centro de Investigación en Sanidad Animal

CISA-INIA, Valdeolmos 28130, Madrid, Spain.

E-mail: eurl.asf@inia.es;

organised by:

EU Reference Laboratory for ASF

Centro de Investigación en Sanidad Animal (CISA-INIA)
Valdeolmos, Spain

and

EU Reference Laboratory for CSF

University of Veterinary Medicine Hannover Institute for Virology Hannover, Germany

In cooperation with

DG Sanco

Tuesday, 03.06.2014.Classical Swine Fever (CSF) session

08:45-09:00 Opening (organisational details).

Francesco Berlingieri(SANCO, EC)
Paul Becher (EURL CSF)

CSF - Session I: "EU Reference Laboratory activities"

Chair: Marisa Arias (ES)

| 9:00-9:30 | Comparative Evaluation of CSF ILCT 2013.S. Austermann-Busch (CSF EURL, DE). |
|-----------|---|
| 9:30-9:45 | Further EURL activities in 2013/14. S. Austermann-Busch (CSF EURL, DE) |

CSF - Session II: "Scientific reports"

Chair: Andrzej Lipowski (PL)

| 9:45-10:05 | Different batches of CSF antibody ELISA: variation in sensitivity. J. Grom (SI) |
|-------------|--|
| 10:05-10:25 | A new concept for the development of a CSF-DIVA ELISA. D. Meyer (CSF EURL, DE) |
| 10:25-10:45 | The Lapinized Chinese vaccine strain against CSFV. Sterilizing protection or viral evolution promoting? L. Ganges (ES) |
| 10:45-11:15 | Coffee break |
| 11:15-11:35 | Alternative sampling strategies in wild boar, A. Petrov (DE) |
| 11:35-12:05 | Welfare issues of laboratory swine used in experimental units for CSF research. K. Marinou (GR) $$ |
| 12:05-12:25 | Genetically modified viruses as vaccine candidates – Lessons learned from a chimeric Pestivirus. A.Postel (CSF EURL, DE) |
| 12:25-12:45 | Rescue of the CSFV Koslov strain from a cloned cDNA, T.B. Rasmussen (DK) |
| 12:45-13:00 | Classical swine fever in wild boars: Demonstration of CSF freedom in Northern Vosges - France. M.F. Le Potier (FR) |
| 13:00-14:00 | Lunch |
| 14:00-14:20 | Definition of genogroup of classical swine fever virus isolates by HRM-analysis.I.A. Titov (RU) |

CSF Session III: "EU projects and working groups"/ invited experts

Chair: Joze Grom (SI)

| 14:30-15:00 | CSFV_goDIVA: Update on CSF marker vaccine. F. Koenen (BE) |
|-------------|--|
| 15:00-15:30 | EURL CSF WB database for CSF surveillance in wild boar. C. Staubach (DE) |

CSF session IV: "Country reports: EU Member states and third countries"

Chair: Helen Crooke (UK)

| 15:30-15:45 | Current CSF situation in Latvia, R. Granta (LV) |
|----------------------------|--|
| 15:45-16:00 | Laboratory diagnostics and epizootic situation for classical swine fever in the Republic of Belarus, O. Mezhennikova (BY) |
| 16:00-16:30 | Coffe break |
| 16:30-16:45 | CSF situation in the Republic of Serbia, V. Milicevic (RS) |
| 16:45-16:55 16:55-17:00 | CSF situation in the Republic of Macedonia 2014 – Country report, Diagnostic Capacity and Structure of the NRL for CSF, I. Djadjovski (MK) CSF situation in Montengro, B. Adzic (ME) |
| 10.55-17.00 | CSF Situation in Monterigro, B. Adzic (ML) |
| 17:00-17:30 | Final discussion on conclusions and recommendations |
| 17:30 | Closure of the workshop |

Rescue of the CSFV Koslov strain from a cloned cDNA

Ulrik Fahnøe¹, Graham J. Belsham¹, Dirk Höper², Martin Beer² and <u>Thomas Bruun Rasmussen</u>¹ DTU Vet, Technical University of Denmark, Lindholm, DK-4771 Kalvehave, Denmark
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The classical swine fever virus (CSFV) strain "Koslov" is highly virulent with a mortality rate of up to 100% in pigs. In this study, we have generated full-length CSFV cDNA clones starting from the blood of Koslov virus infected pigs using a strategy that enables the production of numerous full-length cDNA clones directly from the viral RNA. However, each cDNA obtained initially was non-functional in terms of infectivity when RNA transcripts were introduced into porcine PK15 cells. Full-length sequencing of the cloned cDNAs revealed deleterious non-synonymous mutations including frames hifts. We therefore modified a non-functional cDNA by site directed mutagenesis, removing the non-synonymous mutations step-by-step, thereby building a consensus Koslov genome sequence at the amino acid level. Virus (vKos) rescued from this consensus cDNA clone displayed similar growth kinetics as the parental Koslov strain when tested in cells. Moreover, vKos was highly virulent when tested in pigs, with infected animals displaying pronounced clinical symptoms leading to high mortality.