SURVEILLANCE PROGRAMS. 
A VIEW FROM THE EXPERIENCE OF 
THE EU AND FAO REFERENCE LABORATORIES

African Swine Fever

Dra. Marisa Arias.

UC DAVIS, CADMS, 19, February, 2015
CENTRE FOR RESEARCH ON ANIMAL HEALTH
INIA-CISA, Valdeolmos,
Launched in 1993

Research and development new tools and technologies for prevention and control of Emerging and Transboundary animal infectious diseases of high economic impact. International Cooperation and Technology Transfer

INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria
1993: INIA-CISA, Valdeolmos

BIOSAFETY LEVEL 3 and 4 (Agri) FACILITY
40 BSL-3 laboratories.

2 BSL-4 (Agri)

- CISA Valdeolmos -
BSL-3 ANIMAL FACILITY

19 BSL-3 Animal Rooms
Two Corridors
Pneumatic doors
Differential pressure
AFRICAN SWINE FEVER

EU REFERENCE LABORATORY, 2002-to date

FAO REFERENCE CENTRE, 2013-to date
AFRICAN SWINE FEVER

EU REFERENCE LABORATORY, 2002-to date

FAO REFERENCE CENTRE, 2013-to date

AFRICAN SWINE FEVER

NETWORK OF NATIONAL REFERENCE LABORATORIES FOR ASF

ASF/CSF WORKSHOPS
TRAINING COURSES, EU MS and Eastern European countries.

2010: Estonia, Latvia, Lithuania, Poland, Bulgaria, Romania, Hungary, Sweden, Austria, Slovakia, Slovenia, Czech Republic, Ireland, Italy and Cyprus.
Short-Term Trainings

EU NRLs: Netherlands, Poland, Italy, Greece, Croatia...

Russia and Belarus
EPI-LAB training course, CISA/UCM, June, 2011

...from 2011

Belarus,
January 2011, 2013

Ukraine,
June 2012, 2013

Russia

NRLs Eastern Countries: Russia, Belarus, Ukraine,
NRLs ASIA: China, Vietnam.
IMPROVING KNOWLEDGE OF THE DISEASE, and TECHNOLOGY TRANSFER ON SITE. Training courses on “African swine fever (ASF) diagnostic”

- CVET Missions: PL, Lith, Latvia, 2014

Next: .. Eastern Europe and China

...DEVELOPING ASF DIAGNOSTIC SKILLS TO THE NATIONAL REFERENCE LABORATORIES

...In China 2011 (Lanzhou, NRL Kingdao)
THREAT OF ASF SPREAD IN EASTERN EUROPE: URGENT NEED FOR INTERNATIONAL COLLABORATION.
Budapest, December 4-5 2012

RESOLAB). Sixth Annual Coordination Meeting, Dakar, Senegal, December 3-7 2012


EXPERT WORKSHOP ON ASF, GLOBAL RESEARCH ALLIANCE, PLUM ISLAND (PIADC), N.YORK, USA, April 2013, SouthAfrica, Nov, 2014,

ASF GLOBAL ALLIANCE, ROME, ITALY FAO Headquarters November, 2013,

AU-IBAR, FAO, EAST AFRICA ASF WG, Tanzania 2015.
ASF CURRENT SITUATION

- INFECTIOUS DISEASE with an ongoing spread in Africa & Europe
- Complex epidemiological situation, with reservoirs, and DIFFERENT SCENARIOS with different ASF virus circulating.
- Presence of carrier domestic animals (inapparent, recovered, with virus presence in tissues) in endemic areas which play a role in virus spreading.
- Complex virus.

NO VACCINE

- Causative agent of ASF is not an “unique” virus: We should talk about a “family” of virus (multigenic families).
AFRICAN SWINE FEVER

- Working on surveillance and control: A view from the experience of the EU and FAO Reference Laboratories.
- Advances in Diagnosis, research in progress and some needs
ASF PREVENTION AND CONTROL

CONTROL OF THE DISEASE IS MAINLY BASED ON DISEASE EARLY DETECTION AND THE APPLICATION OF STRICT SANITARY MEASURES

Recognition of the disease in the field

Laboratory Diagnosis

LABORATORY DIAGNOSIS IS ESSENTIAL FOR THE CONTROL OF ASF (THOUGH NOT ENOUGH)
To improve detection it is necessary wider knowledge of clinical presentations.
Improving knowledge about the epidemiological situation of ASF in Africa

Description of the epidemiological situation in African countries based on epidemiological findings and sample collection.

Sampling and characterisation of currently circulating field strains

Improve understanding of virus spread and maintenance in West and east African countries
2004-2011 Epidemiology and prevalence study of African Swine Fever in Kenya and Uganda

### Surveillance program in Kenya and Uganda

By sampling collection in domestic pigs, wild suids, ticks.

Diagnosis and characterization of ASFV isolates

#### Prevalence study of ASFV in wild pigs (warhogs, bushpigs) and ticks (Kenya) and their role in the transmission of the disease.

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<td></td>
<td>2009 TOTAL</td>
<td>0</td>
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<td>TOTAL KENYA</td>
<td>195</td>
<td>379</td>
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<table>
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<td>UGANDA</td>
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<td></td>
<td>2004 TOTAL</td>
<td>0</td>
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<tr>
<td></td>
<td>2007 TOTAL</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL UGANDA</td>
<td>0</td>
<td>316</td>
</tr>
</tbody>
</table>
ASF circulating viruses in Africa

IN AFRICA WILDSUIDS PERSISTENTLY INFECTED FOR LONG PERIODS. NO CLINICAL SIGNS

What is the clinical picture we should expect in domestic animals?
CLINICAL AND PATHOLOGICAL CHARACTERIZATION OF AFRICAN STRAINS BY IN VIVO EXPERIMENTS

ASFV circulating isolates from circulating strains, particularly those exhibiting major variation is eastern African countries.

- ASFV isolates from Eastern Africa (Kenya):
  - Domestic cycle (genotype IX):
    - Viremia: from 4-7dpi; High virulent. No Ab response, or in low % before died in the second week pi
  - Sylvatic cycle (genotype X):
    - Viremia: from 4-7dpi
    - Pigs died between 11-21 (CC)dpi
    - Two pigs (C7,C8)survive to infection
MAIN FINDINGS with circulating viruses in East Africa. Studies in EUROPEAN BREEDS

✓ ACUTE FORM of the disease showing typical clinical signs and lesions associated to ASFV acute strains. Viremia positive, low percentage or not antibody response.

✓ SUBACUTE FORM of the disease showing typical clinical signs and lesions associated to ASFV moderate strains.
  - Viremia detectable by OIE-prescribed virological diagnostic techniques at early times post infection and was maintained during the whole infection.
  - Antibody response detectable by OIE-prescribed serological diagnostic techniques developed in the second week of infection.
EAST AFRICAN REGIONS:
DOMESTIC PIGS: Non evident ASF clinical signs in ASF outbreaks in domestic pigs in combination to a lack of humoral response (low seroprevalence) co-existing with a high viral load (40% of sampling).

Are the diagnostic techniques sensitive enough?
What about the virus?
What about the breeds?....
Are the diagnostic tools adapted to the different scenarios?
Evaluation of serological diagnostic tools in epidemiological situations of Europe, west and eastern Africa.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Country of origin</th>
<th>Host Species</th>
<th>Year of outbreak</th>
<th>P72 genotype</th>
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<tr>
<td>E70</td>
<td>Spain</td>
<td>Domestic pig</td>
<td>1970</td>
<td>I</td>
</tr>
<tr>
<td>Armenia</td>
<td>Armenia</td>
<td>Domestic pig</td>
<td>2007</td>
<td>II</td>
</tr>
<tr>
<td>Moz64</td>
<td>Mozambique</td>
<td>Domestic pig</td>
<td>1964</td>
<td>V</td>
</tr>
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<td>MwLil 20/1</td>
<td>Malawi</td>
<td>Domestic pig</td>
<td>1983</td>
<td>VIII</td>
</tr>
<tr>
<td>Ken06.Bus</td>
<td>Kenya</td>
<td>Tick</td>
<td>2006</td>
<td>IX</td>
</tr>
<tr>
<td>Ken08Tk.2/1</td>
<td>Kenya</td>
<td>Ornithodorus porcinus</td>
<td>2008</td>
<td>X</td>
</tr>
</tbody>
</table>

STRATEGY: to develop New serological diagnostic tools (ELISA and IPT as confirmatory test using new Antigens obtained from a number of virus isolates of several specific genotypes.
1. Analysis of **1,062 FIELD SERUM** samples collected from different epidemiological situations since 2003-2010 from both domestic and wild pigs of Africa and Sardinia.

2. **221 negative field serum samples** from free areas of Europe.

3. Analysis of **214 experimental serum samples** from pigs inoculated with different genotypes (I, II, IX, X) at CISA and IZS-Italy.

1. A panel of **778 Negative field serum samples from ASF virus positive domestic pigs from eastern african countries showing not clinical signs was included**.
The current ASF serological diagnostic tools are adapted to all epidemiological situations.
Comparative study of ASF in INDIGENOUS and EUROPEAN domestic pigs.

Clinical and pathological characterization of Kenyan isolates.

In collaboration with the Kenyan Vet Services

29 Indigenous domestic swine (local breed) from Homa Bay district (6-month old)

10 Exotic domestic swine (Landrace) from Kitengela (6-month old)
Selected ASF virus → Ken05/K2

ASFV belonging to the associated-sylvatic cycle **genotype X** derived from a **domestic pig** from Kiambu district isolated from the Farmer’s Choice slaughterhouse, in Nairobi, **Kenya**.

The animal was **sero-negative but PCR and virus positive** and according to Farmer’ Choice veterinarians appeared **asymptomatic prior to slaughter**.
Comparative $T^a$ (average)

Daily control of clinical signs and temperature (clinical score).
At 14dpi 16 out 29 HB pigs gave a positive result on virus isolation with titers around $10^5$, same titer than in LD pigs.

**ANTIBODY RESPONSE:**

✓ A delay in the seroconversion in indigenous pigs. At 28 dpi 66% did not present antibody response.
VIRUS IN TISSUES LOCAL PIGS VERSUS LANDRACE PIGS

**SPLEEN**

- LD SP
- HB SP

**LYMPHNODES**

- LD SP
- HB SP

RTA2011-00060 SPANISH GRANT
Different behaviour mainly related to the clinical course of ASF.

Significant Delay of onset of ASF in “local breeds” (incubation period).

Similar gross lesions. Vascular changes were more intense in Local breeds (subacute clinical form of ASF) than in Landrace pigs (acute clinical form of ASF).
ASF circulating viruses in East Europe

What we could expect in domestic animals, backyard pigs and wildboar?
“In vivo” STUDIES . BIOLOGICAL CHARACTERIZATION REPRODUCTION OF THE DISEASE.

Azerbaijan: Az08D
Armenia: Arm 07
Ukraine: Ukr12/ZAPO

✓ VIRULENT STRAINS
✓ ACUTE FORM OF THE DISEASE
✓ HIGH MORTALITY: VERY DEPENDENT of the ROUTE, and DOSES. usually from 6 -9dpi, in Domestic Pigs

✓ ANTIBODY RESPONSE: from second week of infection, few animals
Experimental “in vivo” study LT14/1490

- 8 Landrace x Large White pigs inoculated by the intramuscular route with 10 HAD50/ml of LT14/1490
- 10 in-contact pigs.

Similarities with clinical pictures observed at Idavang, big farm, 20,000 pigs dead or killed.

LITHUANIA, outbreak, July, 2014
Experimental “in vivo” study LT14/1490

**Dynamic of infection. Clinical course: Acute infection.**

**newly infected:** incubation period 4-5 days clinical signs appearance and finally dead or moribund around 8-18 dpi.

**In-Contact animals:** clinical disease appearance not before 12-14 dpi after virus infection in progress in the vicinity. Dead or moribund from 15-23 dpi.

At necropsy: High amount of ASFV in tissues.

-Viremia
- Abs: 33% animals
Lithuania, Idavang pig farm, Rupinskai, July 2014 Outbreak

Mortality in the weaner Unit

Source: CVO, Lithuania, August 2014, SCofAH
Mortality in the fattenning Unit

Great wave of deaths

Mortality in the sows Unit

Great wave of deaths

Source: CVO, Lithuania, August 2014, SCofAH
Clinical examination & laboratory tests
At Idavang the animal keepers and veterinarians observed the typical symptoms and signs of the acute form of ASF. The findings highlights that thorough CLINICAL EXAMINATIONS OF PIGS IS A VERY IMPORTANT MEASURE IN THE DETECTION OF ASF.

It is recommended that any suspicion of ASF should result in immediately submission of samples for laboratory testing.

Source: CVO, Lithuania, August 2014, SCofAH
ASF circulating viruses in Europe

WHAT ABOUT CARRIER ANIMALS? ARE THEY WELL RECOGNISED? COULD CARRIER TRANSMIT THE VIRUS?..
ADVANCES IN CARRIER STUDIES.

TRANSMISSION DATA OF PIGS RECOVERED FROM LOW VIRULENT ISOLATES

NHV P68

$10^5_{TCDI/50}$ (1ml/pig) dpi

<table>
<thead>
<tr>
<th>0</th>
<th>35</th>
<th>65</th>
<th>134</th>
</tr>
</thead>
</table>

4.5 months
END OF THE EXPERIMENT

Slaughtered Studies for virus presence in tissues

TWO CONTACT PIGS
Get infected by ASFV

- VIRUS TRANSMISSION FROM CARRIER pigs (don´t showing clinical signs) TO CONTACT PIGS OCCURRED AFTER 2.5 months,

- CARRIERS: VIRUS IN TISSUE TILL 99 DPI.
RECOGNITION OF ASF IN THE FIELD

KEEP UPDATING THE KNOWLEDGE OF CLINICAL PRESENTATIONS

UPDATE EPIDEMIOLOGICAL INFO
Vet. Authorities,
Veterinarians,
Farmers,
Diagnosticians,...
Control of the disease is mainly based on disease early detection and the application of strict sanitary measures.

Laboratory diagnosis is essential for the control of ASF (though not enough).
**LABORATORY DIAGNOSIS**

**Virus detection techniques**

(A) DETECTION OF THE VIRUS GENOME:
- PCR

(B) DETECTION OF VIRUS ANTIGENS
1. Direct immunofluorescent test (DIFT)
2. ELISA for antigen detection

(C) VIRUS ISOLATION AND IDENTIFICATION BY THE HAEMADSORPTION TEST (HAD)

**Antibody detection techniques**

(A) SCREENING BY ELISA
1. OIE-ELISA (Indirect ELISA)
2. COMMERCIAL ELISAs

(B) CONFIRMATORY TESTS
1. Immunoblotting test (IB)
2. Immunoperoxidase test (IPT)
3. Indirect Immunofluorescence test (IFI)
<table>
<thead>
<tr>
<th>AVAILABLE TESTS</th>
<th>TYPE, In house/ Commercial</th>
<th>Recommended Use</th>
<th>REFERENCE</th>
</tr>
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<tbody>
<tr>
<td>Virus Isolation</td>
<td>*VI /Haemadsorption (HAD) test (i.h.)</td>
<td>Confirmation of primary outbreak.</td>
<td>Malmquist and Hay, 1960</td>
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<td>Antigen detection</td>
<td>*Direct Immuno fluorescence (FAT) (i.h.)</td>
<td>Individual testing</td>
<td>Bool et al., 1969</td>
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<tr>
<td></td>
<td>ELISAIngezim-K2, Double AbSandwich/ Commercial</td>
<td>Surveillance Herd testing</td>
<td>INGENASA</td>
</tr>
<tr>
<td></td>
<td>ELISA (i.h.)</td>
<td>Not in use</td>
<td>Pastor et al.1990; Hutchings and Ferris, 2006;</td>
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<tr>
<td>PCR</td>
<td>Conventional (i.h.)</td>
<td>Surveillance Individual and Herd testing</td>
<td>*Aguero et al. 2003.</td>
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<td>Multiplex ASF-CSF (i.h.)</td>
<td>Co-circulation ASF and CSF</td>
<td>Aguero et al. 2004.</td>
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<tr>
<td>Real Time</td>
<td>Taqman Probe (i.h.)</td>
<td>Surveillance Individual and herd testing</td>
<td>*King et al., 2003; *Zsack et al. 2005; Tignon et al. 2011</td>
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<td>UPL Probe (i.h.)</td>
<td>Surveillance Individual and herd testing</td>
<td>Fernandez-Pinero et al. 2013</td>
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<td>MGB Probe (i.h.)</td>
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<td>McKillen et al., 2010</td>
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<td>TETRACORE dried down (Commercial )</td>
<td>Surveillance Individual and herd testing</td>
<td>TETRACORE</td>
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<tr>
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<td>Haines et al.2013</td>
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<td>Isothermal Tests</td>
<td>Invader Assay</td>
<td>Not in use</td>
<td>Hjertner et al., 2005</td>
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<td></td>
<td>LAMP assay</td>
<td>Not in use</td>
<td>James et al., 2010</td>
</tr>
</tbody>
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*Included in the OIE Terrestrial Manual for Diagnostic Test and Vaccines, 2012.*
**AVAILABLE DIAGNOSTIC TESTS**

**ANTIBODY DETECTION TECHNIQUES**

<table>
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<th>AVAILABLE TESTS</th>
<th>TYPE, In house/ Commercial</th>
<th>Recommended Use</th>
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<td><strong>ELISA Tests</strong></td>
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<td>*OIE Indirect ELISA (i.h.)</td>
<td>Surveillance Herd testing</td>
<td>Sánchez-Vizcaíno et al. 1982; Pastor et al., 1990.</td>
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<td>Recombinant proteins (rp)-ELISA (i.h.)</td>
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<td>Gallardo et al. 2006, 2009, Pérez-Filgueira et al., 2006</td>
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<td>ELISA Ingezim-K3, Bloking/Commercial</td>
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<td>Ingezim PPA-CROM Commercial</td>
<td>Surveillance Individual Testing</td>
<td>INGENASA</td>
<td></td>
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<td>Dot Blot (i.h.)</td>
<td>Surveillance Individual Testing</td>
<td>Pastor et al. 1992</td>
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<td><strong>Confirmatory Antibody tests</strong></td>
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</tr>
<tr>
<td>*Immunoblot (IB) Test (i.h.)</td>
<td>Confirmatory Herd testing</td>
<td>Pastor et al. 1989</td>
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<td>*Immunofluorescence Antibody (IFA) test (i.h.)</td>
<td>Confirmatory Herd testing</td>
<td>Pan et al., 1974</td>
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<td>Indirect Immunoperoxidase test (IPT)</td>
<td>Confirmatory Herd testing</td>
<td>Gallardo et al. 2013</td>
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</table>

### Antibody Detection Techniques

- ELISA K3*
- ELISA OIE
- ELISA ID-VET*
- ELISA Svanova*
- PENSIDE test*

### Virus Detection Techniques

- Virus Isolation/HAD
- FAT (DIF) TEST
- Antigen ELISA K2*
- AnKgen ELISA
- FAT (DIF) TEST
- AnKbody Detection Techniques

#### Confirmatory tests:

- IB TEST
- IPT TEST
- IFA TEST
- IB TEST

* COMMERCIAL KITS

### Good Sensitive, Specific and Rapid “In House” and Commercial Techniques

- PCR Tignon
- PCR Tetracore *
- Tetracore/ARS
- Isothermal tests (Not Validated)

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**SUMARIZING AVAILABLE DIAGNOSTIC TESTS**
PENSIDE TEST FOR ASF ANTIBODY DETECTION, validated and commercially available.

**INGEZIM PPA CROM**

**BASE TÉCNICA DEL KIT**
El dispositivo de diagnóstico está compuesto por una placa de plástico con dos ventanas:
- **Ventana de adición de la muestra:** Contiene la proteína VP72 y una proteína control, unidos a partículas de látex coloreado.
- **Ventana de lectura de resultados:** Contiene una línea test (T) formada por proteína VP72 y una línea control (C) formada por un AcM específico de la proteína control.

Al añadir la dilución de la muestra, si contiene anticuerpos, estos se unirán a la proteína VP72 conjugada al látex y migrarán por la membrana. El complejo anticuerpo-antígeno-látex se unirá al antígeno situado en la zona test (T) dando lugar a la aparición de una línea roja/roja. La aparición de una línea azul en la zona control (C) indica que el ensayo es válido.

**APLICACIÓN**
Detección de anticuerpos específicos de la proteína VP72 del Virus de la Peste Porcina Africana, en muestras de suero y sangre porcina.

**SENSIBILIDAD DIAGNÓSTICA**
- 84 sueros de foco negativos procedentes de África Oriental positivos por ELISA OIE.
- 15 sueros de cerdos españoles domésticos positivos por ELISA OIE.
- 109 sueros de cerdos procedentes de África Occidental positivos por ELISA OIE.

99% correspondencia con ELISA OIE.

**ESPECIFICIDAD DIAGNÓSTICA**
- 1043 sueros de cerdos domésticos procedentes de zonas libres de PPA.

**SENSIBILIDAD ANALÍTICA**
- Suero de 13 cerdos experimentalement infectados con diferentes aislados del virus de la PPA.
- Sueros de Referencia de la OIE fuertemente positivo y débilmente positivo.

El ensayo es capaz de detectar anticuerpos entre los días 10 p.i. y 21 p.i., dependiendo del aislado viral:
- Detecta el suero fuertemente positivo de la OIE a dilución 1/64 en suero negativo y el débilmente positivo a dilución 1/2 en suero negativo.
NEW ADVANCES IN DIAGNOSIS 2014

PENSIDE TEST FOR ANTIGEN DETECTION developed and initial validation performed

ADDITIONAL FIELD VALIDATION IS ON-GOING
Overall analysis of the results: More than 240 cases and outbreaks by ASF circulating virus induce Acute infection.

Wild boar: The presence of antibodies was confirmed by IPT test in 60.37% of the wild boar analyzed at the EURL from the affected areas. High antibodies titers in a significant %.

Domestic pigs; The presence of antibodies was confirmed by IPT test in 46.8% domestic pig.
- PCR techniques, very sensitivity for early detection in the epidemic situation. The best sensitivity: UPL-PCR.

**ANTIBODY DETECTION IS VERY USEFUL. TO BE IN MIND:**

- **ASF acute infection: ELISAs** exhibit a limited sensitivity in detection of low antibody titers.

- IPT very sensitive VERY VALUABLE technique, to determine the time of infection.

- **EXUDATE** from tissues VERY VALUABLE SAMPLE in WB.
DIAGNOSIS

what is now coming……
NEW ADVANCES IN DIAGNOSIS 2014

VALIDATION OF NEW SAMPLE TYPES AS AN ALTERNATIVE FOR SAMPLING

SWABS

ASF genome (PCR), virus (virus isolation) and antibodies (ELISA) has been obtained from swabs showing as an useful alternative for passive African swine fever surveillance.

DRIED BLOOD

- Usefulness and feasibility of the filter paper (WHATMAN 3MM) blood collection method for testing of ASF antibodies using the IPT test.
- Usefulness and feasibility of the filter paper (WHATMAN FTA) blood collection method for testing ASF genome (diagnosis and molecular characterization)

ASFV in Tanzania: Asymptomatic pigs harbor virus of molecular similarity to Georgia 2007

Á. Uttenthal 1*, U.C. Braae 2, H.A. Ngowi 3, T.B. Rasmussen 4, J. Nielsen 4, M.V. Johansen 5

1 Section for Virology, National Veterinary Institute, Technical University of Denmark, Frederiksberg, DK-2800, Denmark
2 Section for Parasitology, Health and Development, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2100 Copenhagen, Denmark
3 Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania

* Corresponding author: a.uttenthal@vetsyd.dk
VALIDATION OF NEW SAMPLE TYPES

MEAT JUICE SAMPLES

**use of muscle transudate samples** in ASFV surveillance programs based on **IPT antibody testing and UPL-real time PCR** for ASFV genome detection.

ORAL FLUIDS

Presence of ASFV antibodies in swine oral fluids samples → the possibility of an oral fluid-based approach in ASF diagnosis and, potentially in passive ASF surveillance.
ASF DIAGNOSIS: RESEARCH IN PROGRESS AND FUTURE PRIORITIES
DIAGNOSIS: RESEARCH IN PROGRESS

- Development of **new conventional PCR** based of TK gene for ASF diagnosis with improved sensitivity to the OIE-conventional PCR in the analysis of East African ASFV (genotype II).

- **To Continue assessment of ASF diagnostic tests** taking into consideration the current ASF situation worldwide.

- Validation of **two additional commercial real time PCR assays** coming from INGENASA (UPL-based) and QUIAGEN (King based)

- Development and **initial validation of penside test for Ag detection**

**NEED FOR MORE FIELD SAMPLES FROM AFRICA REGIONS**
Defining New genetic markers to trace the source of the outbreaks and so the dynamic of the disease.

Deeper full genome sequence of currently circulating isolates in Europe is being performed that will allow a better knowledge about virus evolution.

Initial standardization and validation to diagnosis ASF in alternative samples such as exudate tissues, oral fluid, swabs, dried blood samples, meat juice samples.

NEED FOR MORE FIELD SAMPLES FROM AFRICA REGIONS
2. FUTURE PRIORITIES

• Virus isolation techniques need to find cell lines that replace primary cultures.

• Need to develop specific diagnostic tools for ASF detection taking into consideration the worldwide situation (different scenarios, lab capabilities, etc)

• Need to develop high sensitivity ELISAs for the detection of antibodies in alternative samples and for the early detection of the disease.

• Need to intensify virus detection, isolation and characterization from sylvatic cycle hosts in Africa for genotyping purposes.
2. FUTURE PRIORITIES cont.

- Need to increase knowledge of the survivor pigs from the clinical and ASF diagnosis point of view.
- Need to define phylogenetic markers associated with pathogenicity.
- Expand field validation of new developed assays taking into consideration worldwide situation.
- Intensify training and following up activities for international harmonization of ASF current diagnostic tests.
...SOME OTHER DIAGNOSTIC NEEDS

- THERE IS A NEED OF COORDINATION AND FOLLOWING UP ACTIVITIES AT DIAGNOSTIC LABORATORIES

- DIAGNOSTIC INFORMATION UPDATED

- IMPLEMENTATION OF HARMONIZED VALIDATED TECHNIQUES IN NRLS OF THE COUNTRIES

- APPROPRIATE SAMPLING STRATEGY

- PREVALENCE STUDIES in certain regions,

- USE OF VALIDATED TESTS WITH APPROPRIATE SPECIMENS.
A GOOD DIAGNOSIS OF ASF IS ESSENTIAL FOR THE CONTROL OF ASF, BUT NOT ENOUGH, INFORMATION for implementing GOOD ASF CONTROL ESTRATEGIES is needed.
WELCOME TO THE EUROPEAN UNION REFERENCE LABORATORY FOR AFRICAN SWINE FEVER (EURL-ASF).

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)

NEW! ASF REVIEW ARTICLES VIRUS RESEARCH SPECIAL EDITION 2012-2013.

NEW! THREAT OF ASF SPREAD IN EASTERN EUROPE: URGENT NEED FOR INTERNATIONAL COLLABORATION. TECHNICAL MEETING 4-6 DECEMBER 2012, BUDAPEST, HUNGARY

NEW! WORKSHOP ON LABORATORY DIAGNOSIS OF ASF and CSF May 22-June 1 2012, Hannover, Germany.

http://asf-referencelab.info
IT IS THE TIME TO BE READY

ARE YOU READY?
Thanks to the AFRICAN and EUROPEAN TEAMS working with us in ASF and to the

Thank you for your attention

THANK YOU FOR YOUR ATENTION

THANK YOU CADMS

THANK YOU UC DAVIS

EU and FAO REFERENCE
LABORATORIES AND ASF EPIDEMIOLOGY INIA-CISA GROUP