

African Swine Fever

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

Dra. Marisa Arias.



GOBIERNO DE ESPAÑA

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD



UC DAVIS, CADMS, 19, February, 2015

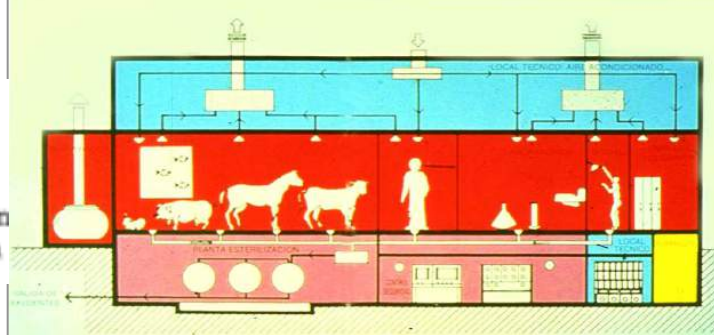


MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD



CENTRE FOR RESEARCH ON ANIMAL HEALTH INIA-CISA, Valdeolmos,

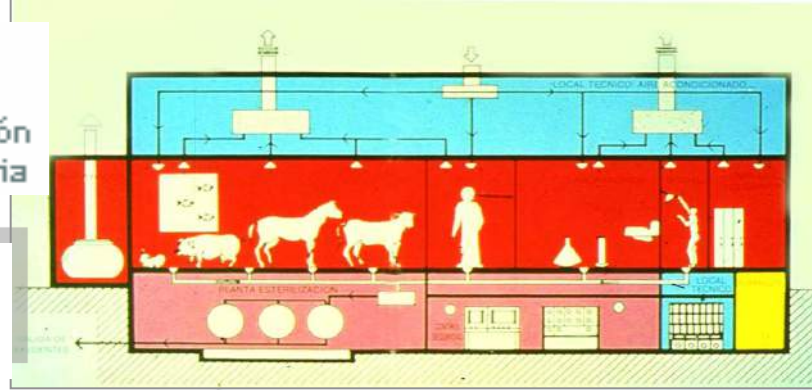




Launched in 1993



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



1993: *INIA-CISA, Valdeolmos*



BIOSAFETY LEVEL 3 and 4 (Agri) FACILITY

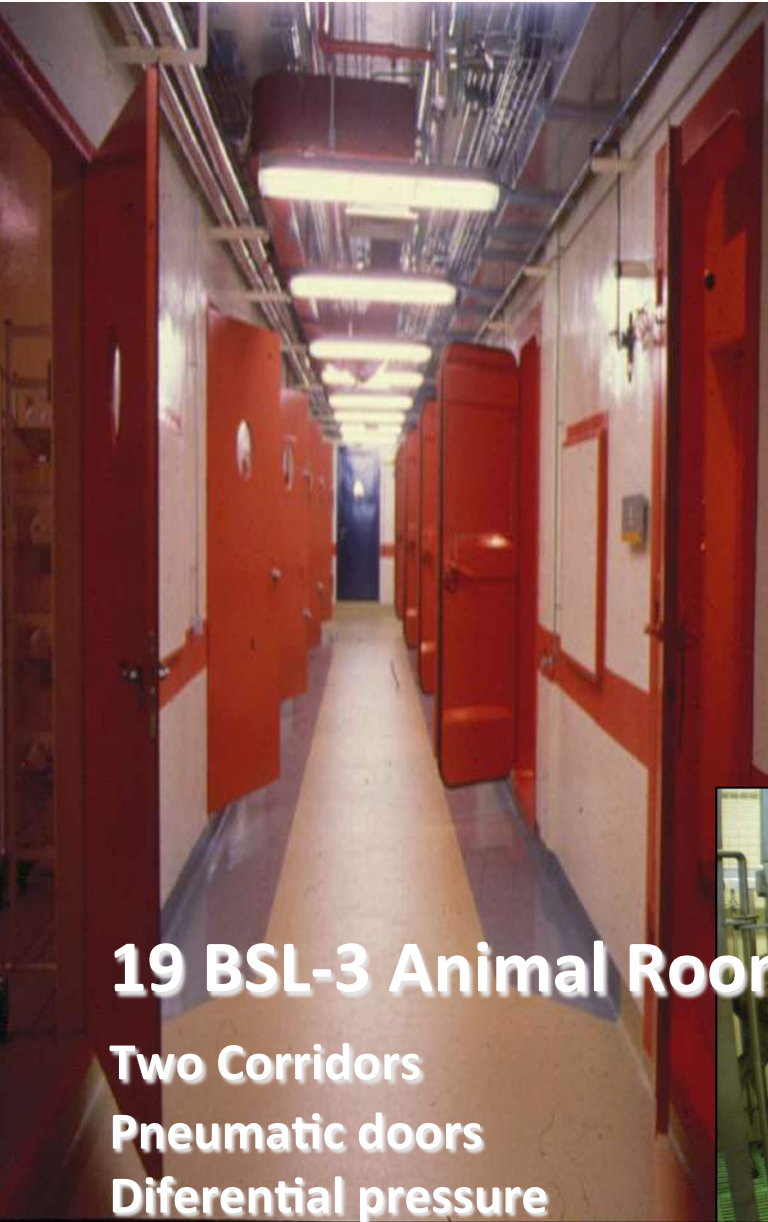


40 BSL-3 laboratories.

2 BSL-4 (Agri)

- CISA Valdeolmos -

BSL-3 ANIMAL FACILITY



19 BSL-3 Animal Rooms

Two Corridors

Pneumatic doors

Diferential pressure



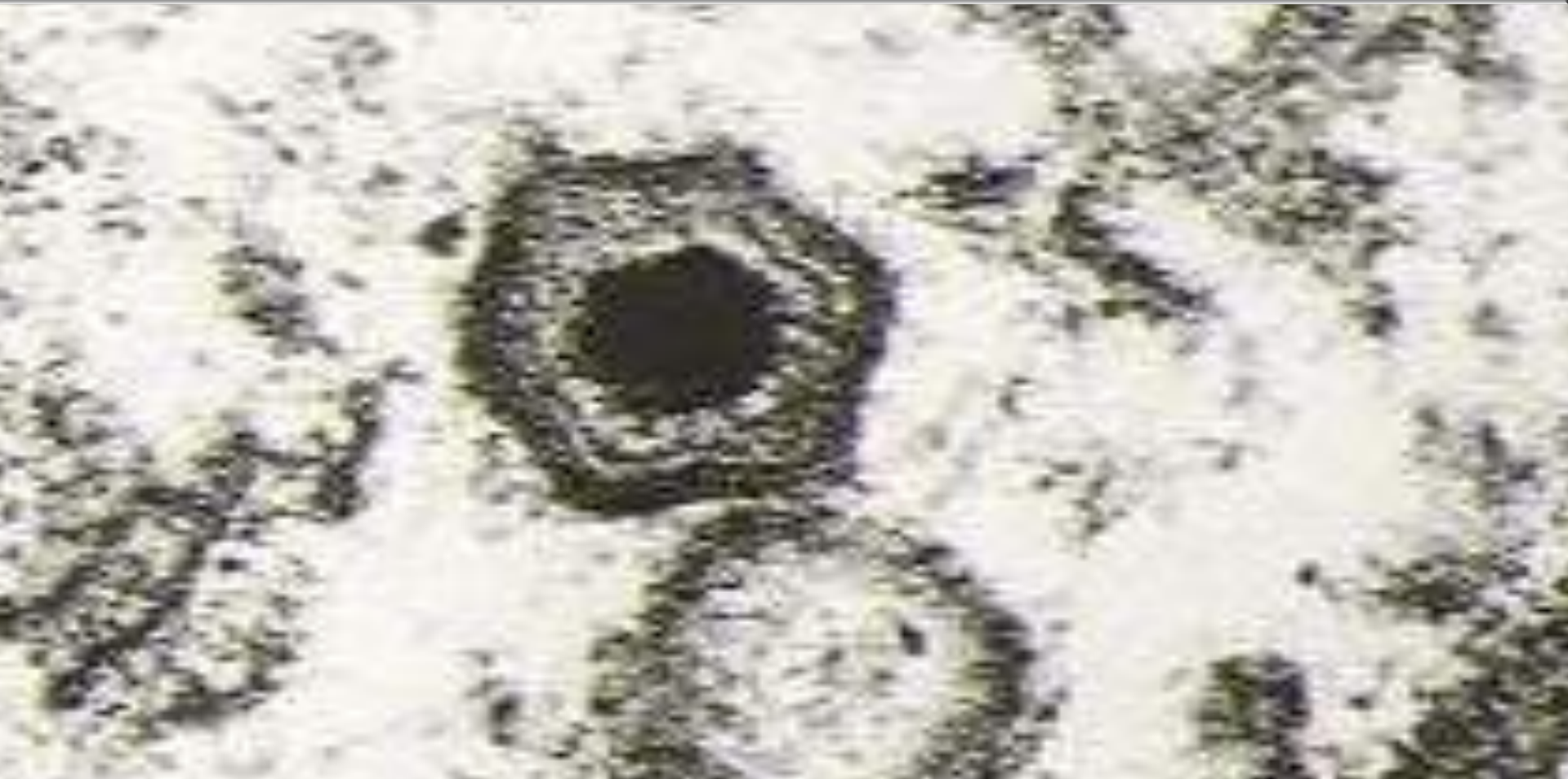
EU REFERENCE LABORATORY, 2002-to date



FAO REFERENCE CENTRE, 2013-to date



AFRICAN SWINE FEVER



EU REFERENCE LABORATORY, 2002-to date



FAO REFERENCE CENTRE, 2013-to date



AFRICAN SWINE FEVER



ASF/CSF WORKSHOPS

**NETWORK OF NATIONAL REFERENCE
LABORATORIES FOR ASF**



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.

TECHNOLOGY TRANSFER



EU Reference Laboratory for ASF
Animal Health Research Centre
(CISA), INIA
Ctra Algete-El Casar s/n
28130, Valdeolmos, Spain



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
June 2008, 2009, 2012



NRLs Eastern Countries: Russia, Belarus, Ukraine, .
NRLs ASIA: China , Vietnam.

MISSIONS ...from 2011



EU Reference Laboratory for ASF
Animal Health Research Centre
(CISA), INIA
Ctra Algete-EI Casar s/n
28130, Valdeolmos, Spain



**IMPROVING KNOWLEDGE OF THE DISEASE, and
TECHNOLOGY TRANSFER ON SITE . *Training courses
on “African swine fever (ASF) diagnostic”***

**TRAINING COURSE ON AFRICAN
SWINE FEVER (ASF) LABORATORY
DIAGNOSIS**

HELD ON
November 14th to 18th 2011 in the National Diagnostic and
Research Veterinary Medical Institute (NDVRI), Bulgaria

AND ORGANIZED BY THE
Centro de Investigación en Sanidad Animal (CISA-INIA)
European Union reference laboratory for ASF
CISA-INIA, Valdeolmos, Madrid, SPAIN
Ctra. Algete-EI Casar, s/n,
Valdeolmos, 28130 Madrid
Tel: +34 91 6202100
Fax: +34 91 6202147



- **NRLs from EU** → **Bulgary (2011), Greece (2012), Lithuania (2014), Poland (2014) .**
- **CVET Missions: PL, Lith, Latvia, 2014**
- **EASTERN EUROPE: RusSia (2011), Ukraine (2011), Armenia (2012) , Kazahjistán (2013) Belarus (2012, 2013)**

...IN EUROPE



Next: .. Eastern Europe and China



MISSIONS : ...*In Africa*



Y COMPETITIVIDAD Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria
International Livestock Research Institute
Better lives through livestock

Kenya (2006, 2007, 2008), Tanzania (2008), Uganda (2009), Nigeria (2009), Ruanda (2010), Burundi (2010/11), SouthÁfrica (2011)...



...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**

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

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



**EU, EFSA, EKE,
EXPERT January,
2014,2015**



**ASF GLOBAL ALLIANCE, ROME, ITALY FAO
headquarters November, 2013,**

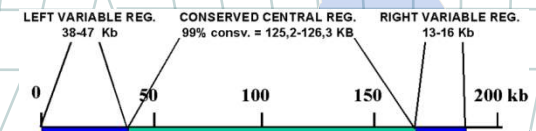
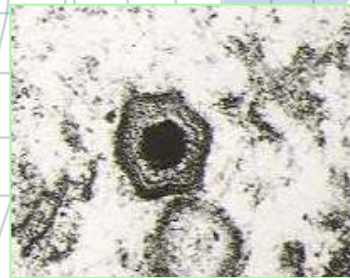


**AU-IBAR, FAO, EAST AFRICA ASF WG,
Tanzania 2015.**



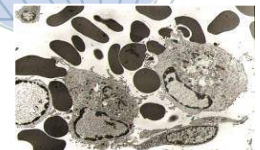
ASF CURRENT SITUATION

- **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** (innaparent, recovered, with virus presence in tissues) in endemic areas which play a role in virus spreading .
- **Complex virus.**



- Causative agent of ASF is not an "unique" virus: We should talk about a "family" of virus (multigenic families).

NO VACCINE





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**

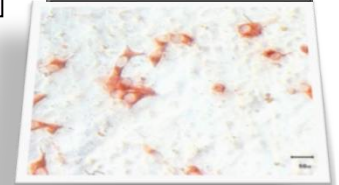
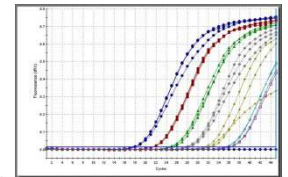
ASF PREVENTION AND CONTROL

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)

RECOGNITION OF ASF IN THE FIELD

...From 2004 working in AFRICA....

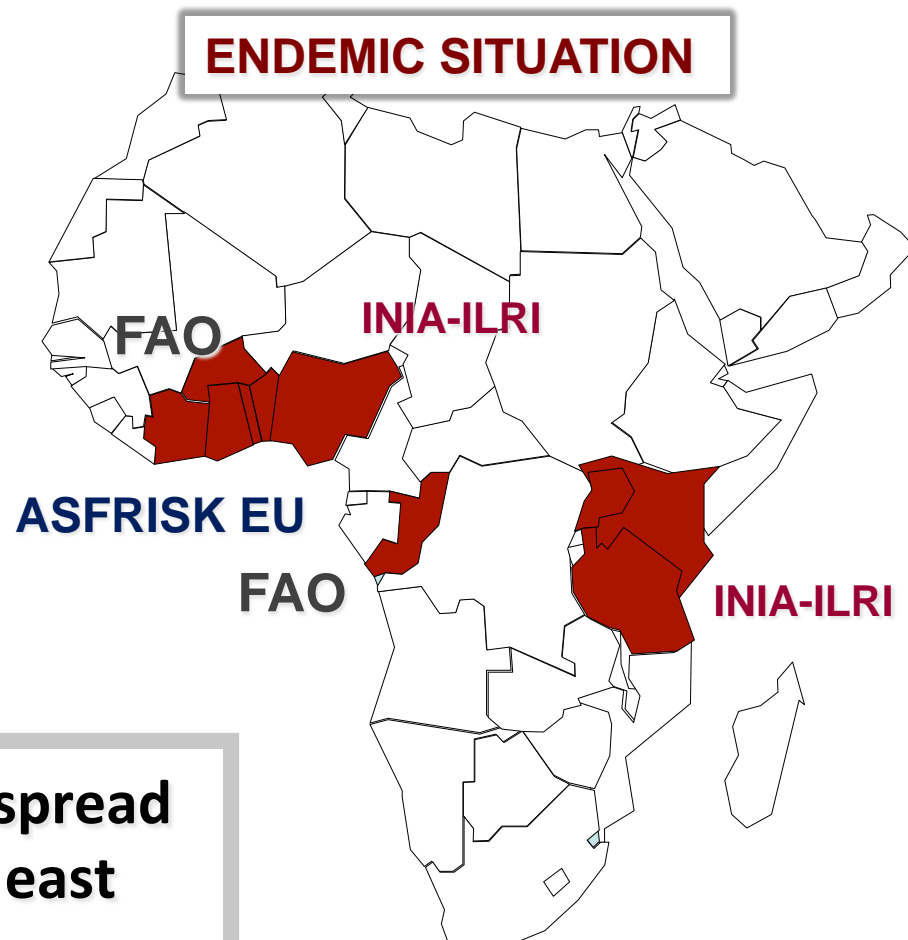
TO IMPROVE KNOWLEDGE ON EPIDEMIOLOGY



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



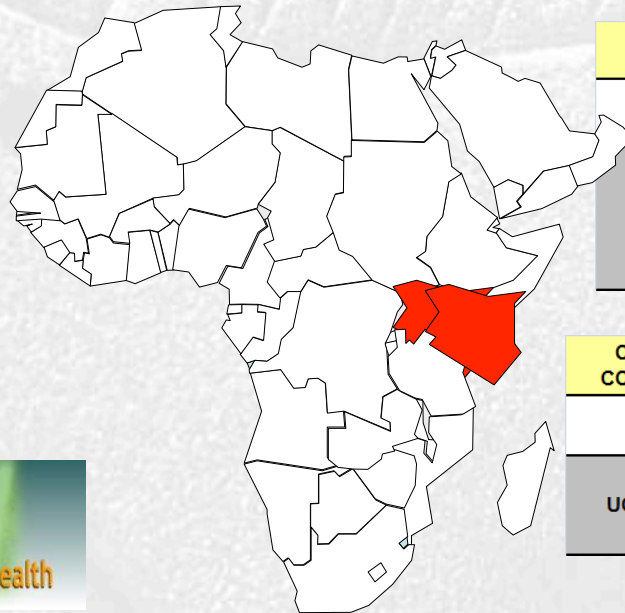
EU Reference Laboratory for ASF
Animal Health Research Centre
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Ctra Algete-El Casar s/n
28130, Valdeolmos, Spain



INIA
Instituto Nacional de Investigación
y Tecnología Agraria y Alimentaria



2004-2011 Epidemiology and prevalence study of African Swine Fever in Kenya and Uganda



ORIGIN COUNTRY	YEAR	TYPE OF SAMPLE				
		FILTER CARDS	SERA	WHOLE BLOOD (EDTA)	TISSUES*	TICKS
KENYA	2004 TOTAL	104	20	50	6	0
	2005 TOTAL	0	86	86	87	365
	2006 TOTAL	9	30	25	7	0
	2007 TOTAL	0	49	25	11	0
	2008 TOTAL	82	88	12	4	28
	2009 TOTAL	0	106	75	14	250
TOTAL KENYA		195	379	273	129	643

ORIGIN COUNTRY	YEAR	TYPE OF SAMPLE				
		FILTER CARDS	SERA	WHOLE BLOOD (EDTA)	TISSUES*	TICKS
UGANDA	2003 TOTAL	0	142	142	6	0
	2004 TOTAL	0	49	49	1	0
	2007 TOTAL	0	125	40	1	0
	TOTAL UGANDA	0	316	231	8	0



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.

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.

CIRCULATING ASFV ISOLATES



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).



Three different
Transmission cycles

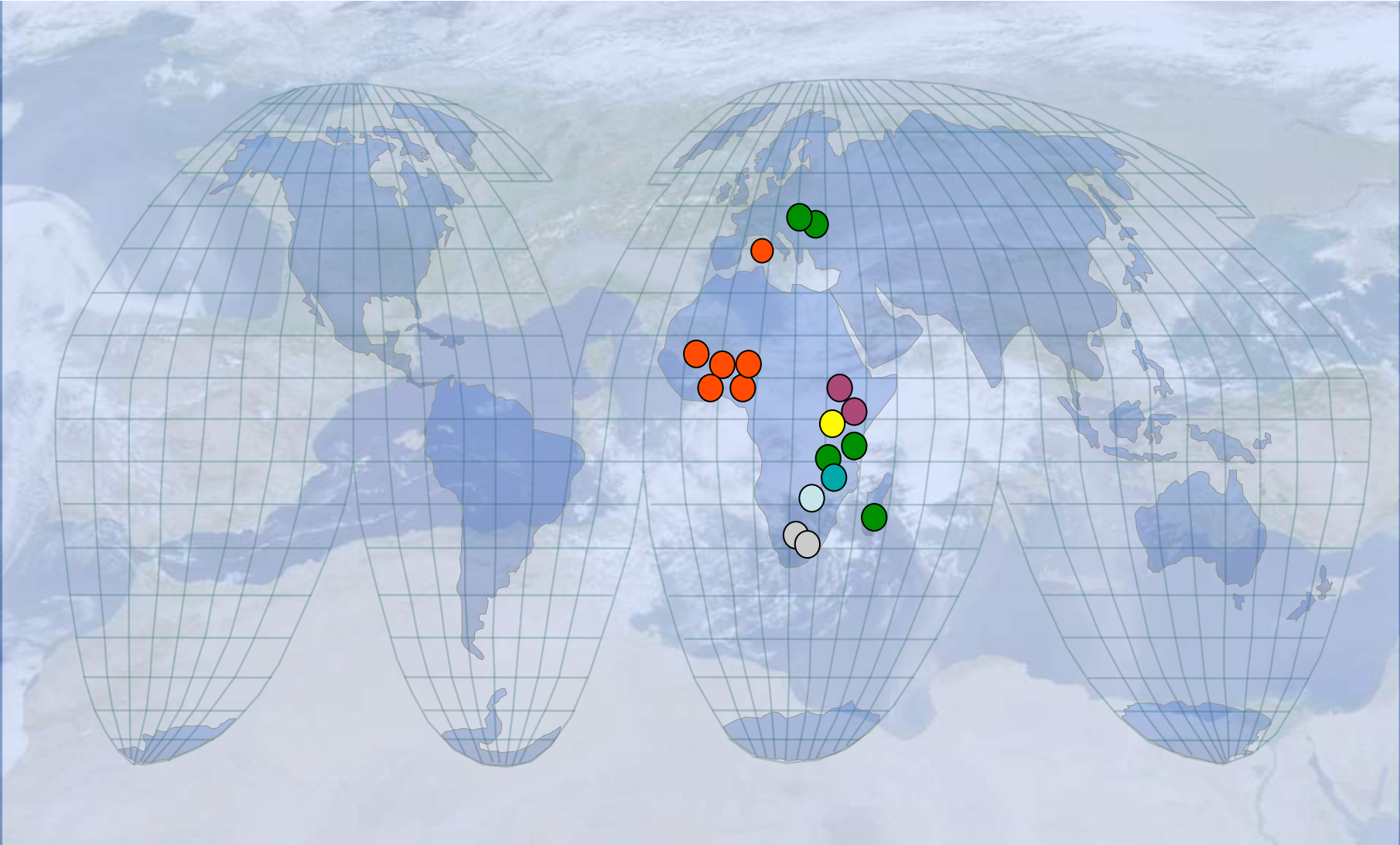
EPIDEMIOLOGY OF ASF VERY COMPLEX.....: East African isolates
↑ GENOME VARIABILITY

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.

Isolate ^a	Country of origin	Host Species	Year of outbreak	P72 genotype
E70	Spain	Domestic pig	1970	I
Armenia	Armenia	Domestic pig	2007	II
Moz64	Mozambique	Domestic pig	1964	V
MwLil 20/1	Malawi	Tick	1983	VIII
Ken06.Bus	Kenya	Domestic pig	2006	IX
Ken08Tk.2/1	Kenya	Omithodorus porcinus	2008	X

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.



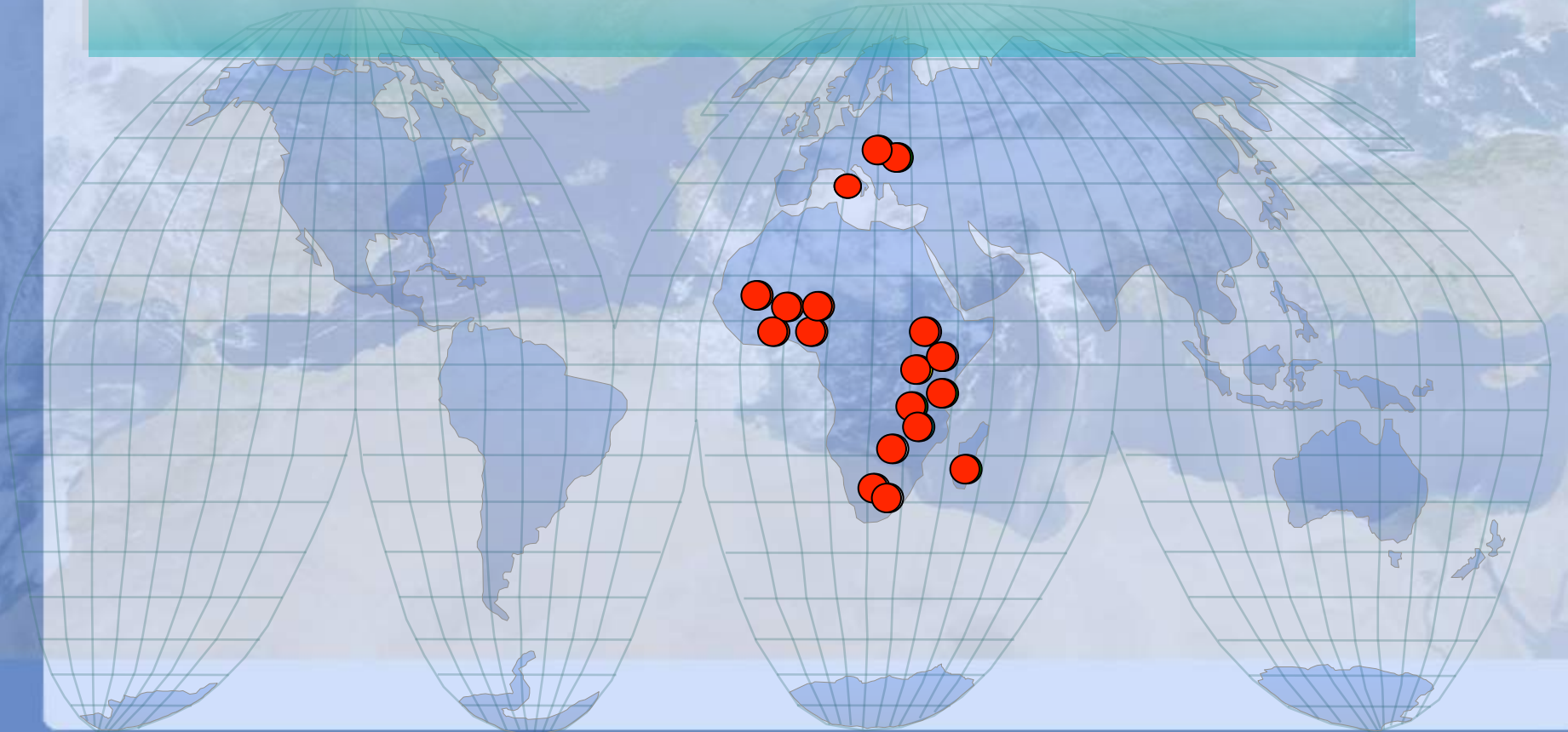
1. A panel of **778 Negative** field serum samples from **ASF virus positive** domestic pigs from **eastern african countries** showing not clinical signs was included .



African Swine Fever



The current ASF serological diagnostic tools
ARE ADAPTED TO ALL EPIDEMIOLOGICAL
and **SITUATIONS**.





ILRI

International Livestock Research Institute

Better lives through livestock



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RTA2011-00060-

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)



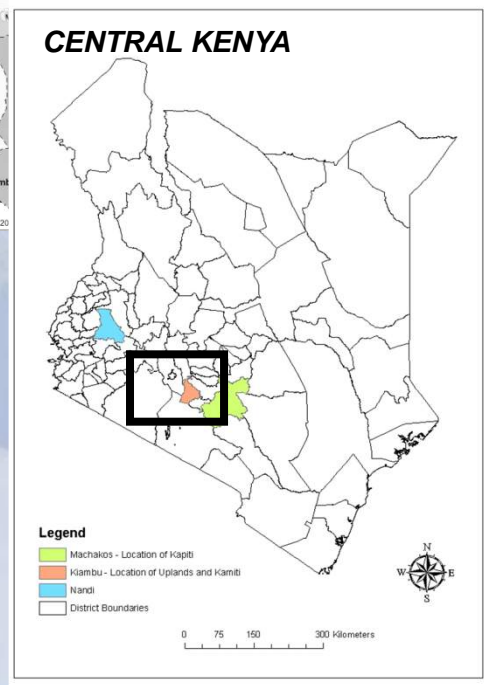
10 Exotic domestic swine (Landrace)
from Kitengela (6-month old)



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**.

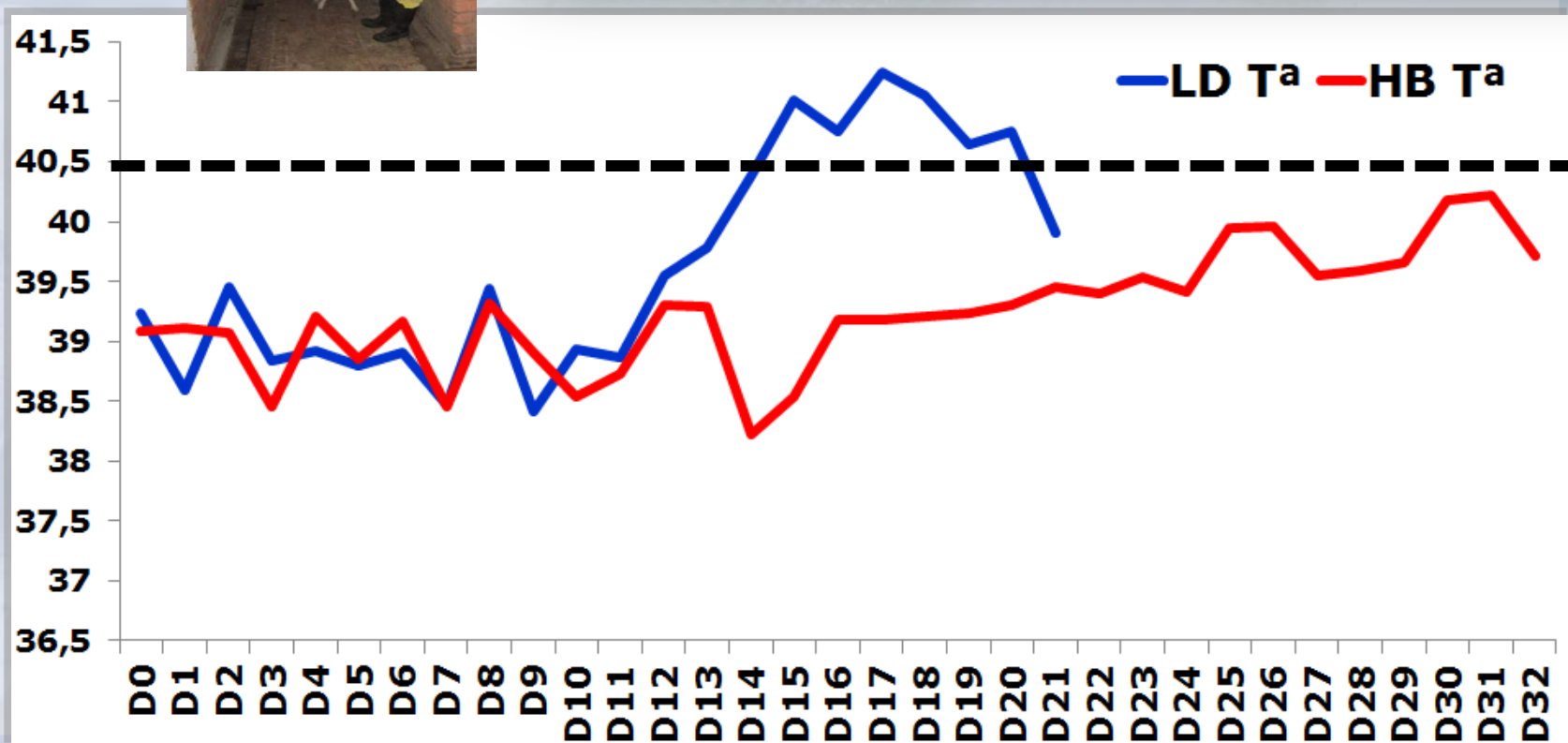
The animal was **sero-negative but PCR and virus positive** and according to Farmer' Choice veterinarians appeared **asymptomatic prior to slaughter**.



COMPARATIVE RESULTS



Comparative T^a (average)

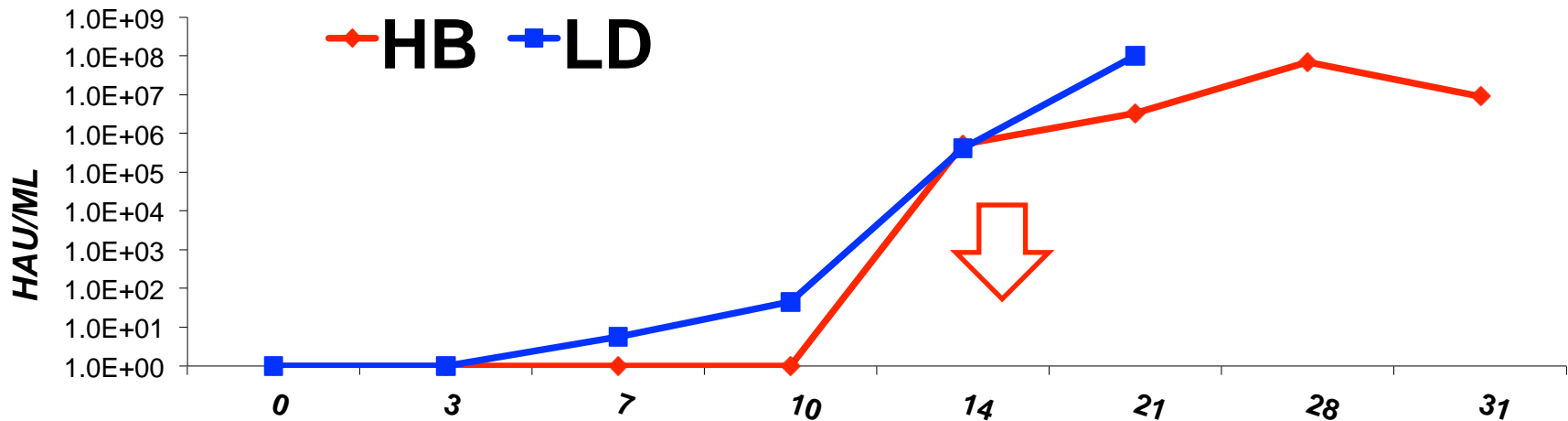


Daily control of clinical signs and temperature (clinical score).

LOCAL PIGS VERSUS LANDRACE PIGS

VIREMIA:

RTA2011-00060 SPANISH GRANT



At **14dpi** **16 out of 29 HB pigs** gave a **positive result** on virus isolation with titers around 10^5 , same titer than in LD pigs.

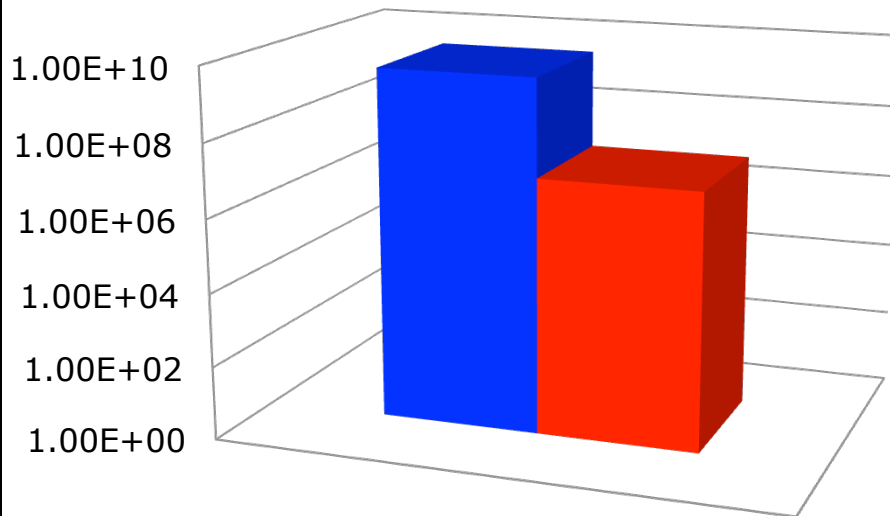
ANTIBODY RESPONSE:

✓ **A delay in the seroconversion in indigenous pigs.** At 28 dpi **66%** did not present antibody response.



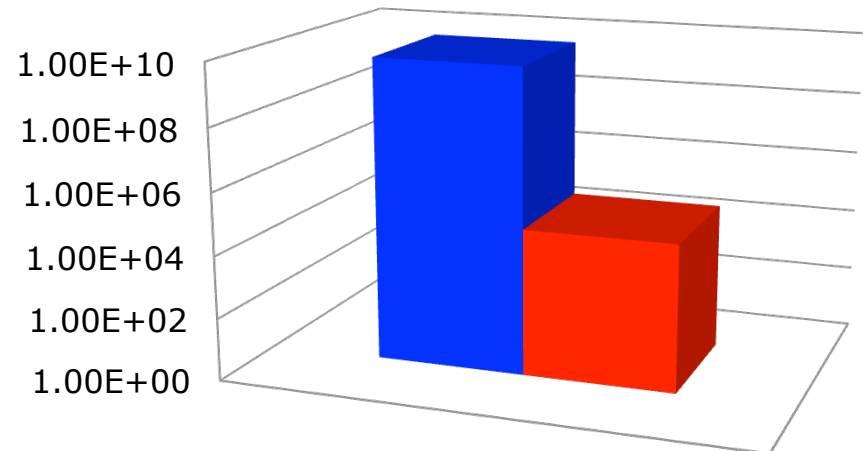
VIRUS IN TISSUES LOCAL PIGS VERSUS LANDRACE PIGS

SPLEEN



■ LD SP
■ HB SP

LYMPHNODES



CONCLUSIONS



EU Reference Laboratory for ASF
Animal Health Research Centre
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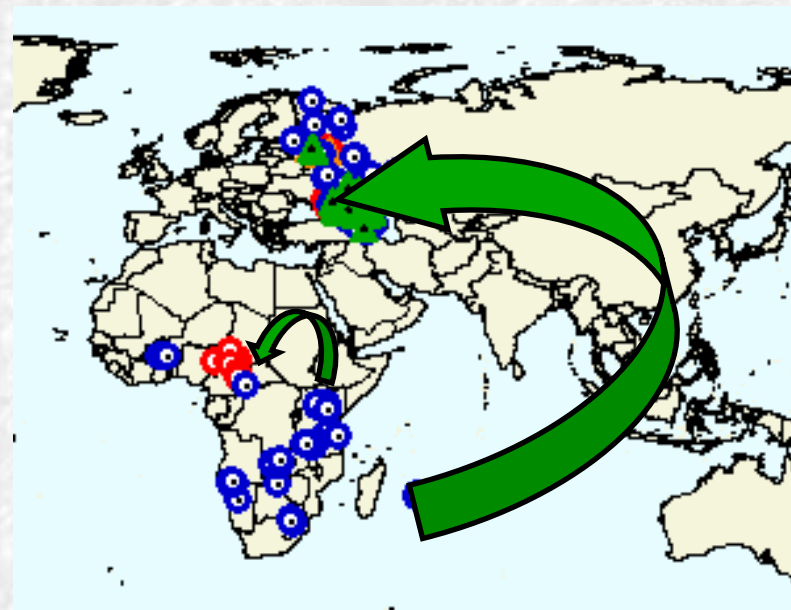
International Livestock Research Institute
Kenia | Tanzania | Uganda

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 (subcute 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



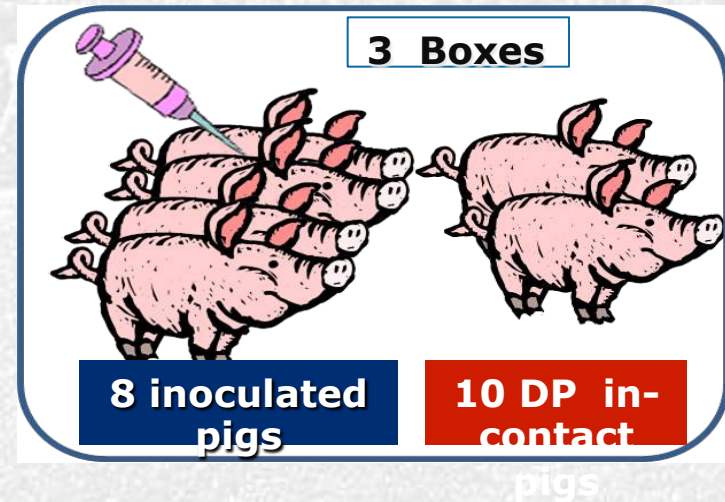
- ✓ **VIRULENT STRAINS**
- ✓ **ACUTE FORM OF THE DISEASE**
- ✓ **HIGH MORTALITY: VERY DEPENDENT of the ROUTE, and DOSES. usually from 6 -9dpi, in Domestic Pigs**

- ✓ **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.**



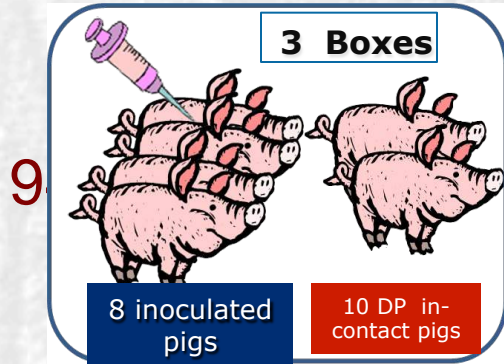
Similarities with clinical pictures observed at Idavang, big farm, 20.000 pigs dead or killed.

LITHUANIA, outbreak , July, 2014



Idavang pig farm, Rupinskai

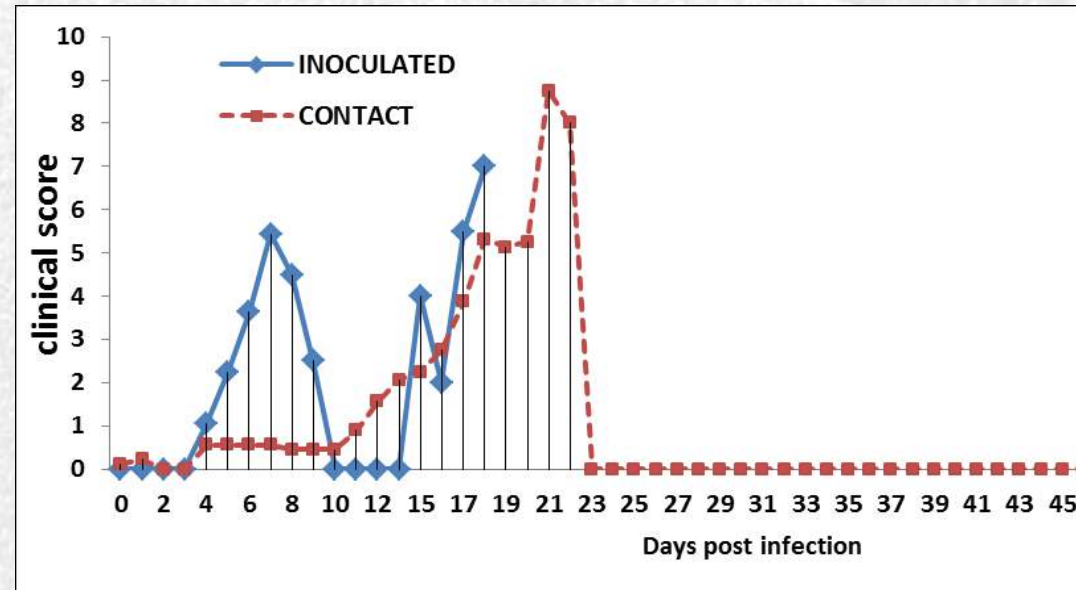
Experimental "in vivo" study LT14/1490



Dynamic of infection.
Clinical course: Acute infection.

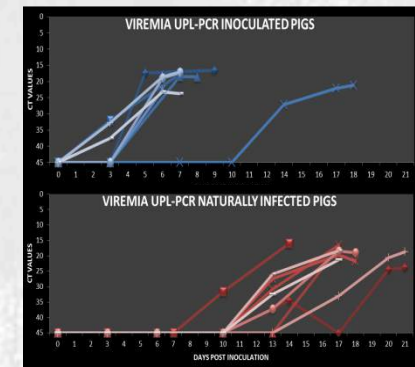
newly infected: incubation period 4-5 days Clinical signs appearance and finally dead or moribund around 8-18 dpi.

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.



-Viremia

- Abs: 33% animals

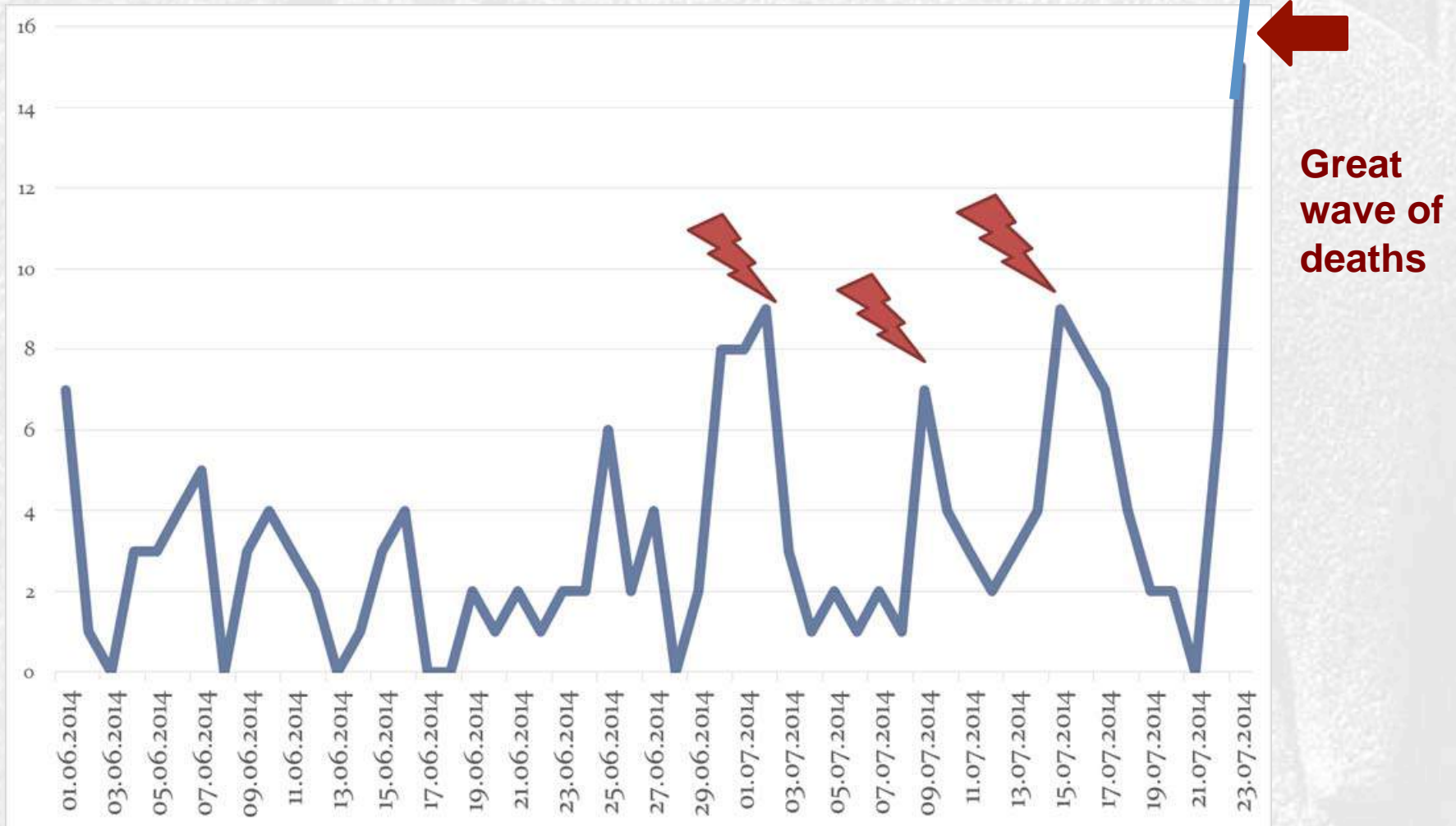


At necropsy: High amount of ASFV in tissues.

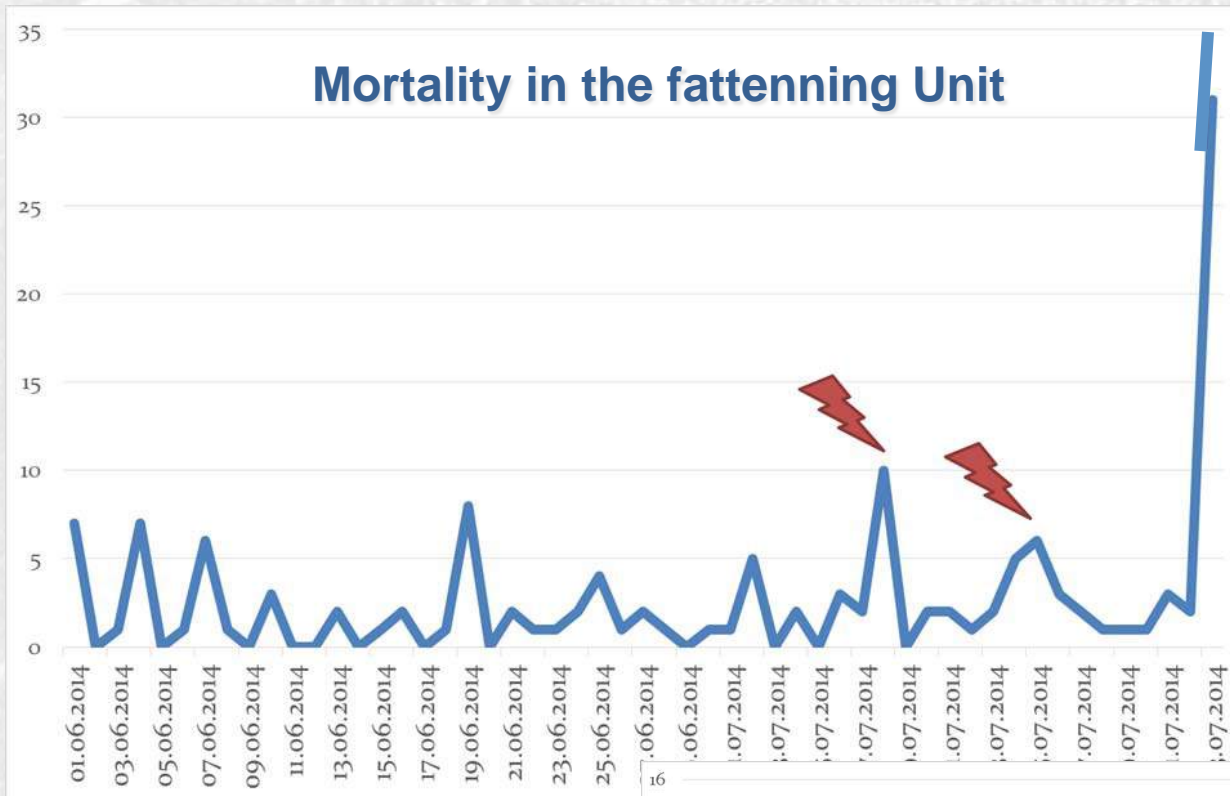


Lithuania, Idavang pig farm, Rupinskai, July 2014 Outbreak

Mortality in the weanner Unit

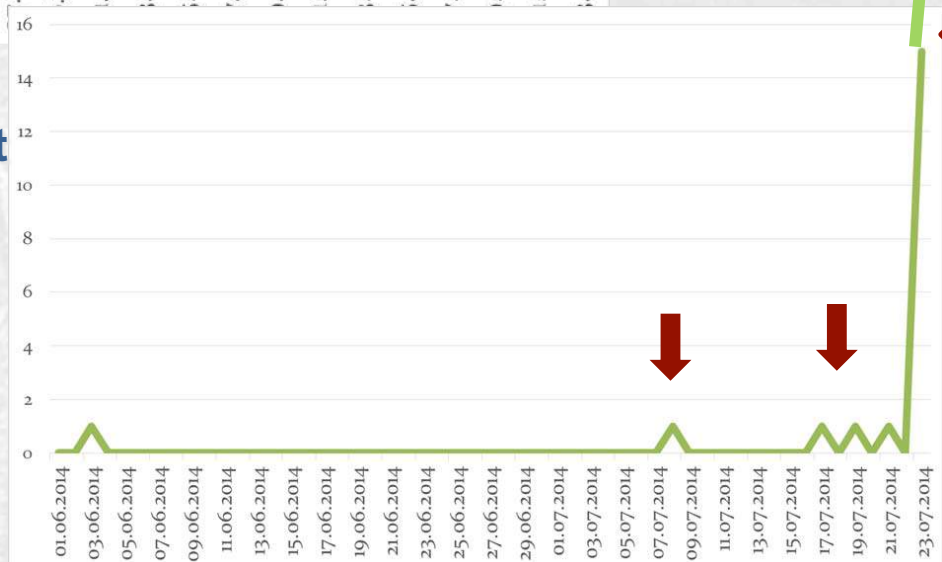


Lithuania, Idavang pig farm, Rupinskai, July 2014 Outbreak



Great wave of deaths

Mortality in the sows Unit



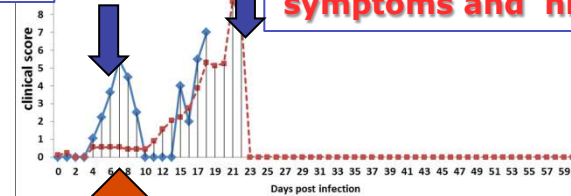
Great wave of deaths



○ **14th -20th July, weaner unit: 18 pigs died**

○ **23-24 July, sows unit: symptoms and high mortality**

RECOMMENDATIONS CVET, EC



Early detection of
clinical signs (holdings)

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.***

ASF circulating viruses in Europe

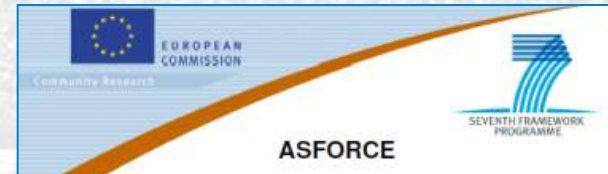
WHAT ABOUT CARRIER ANIMALS? ARE THEY WELL RECOGNISED ? COULD CARRIER TRANSMIT THE VIRUS?..



ADVANCES IN CARRIER STUDIES.



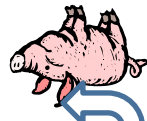
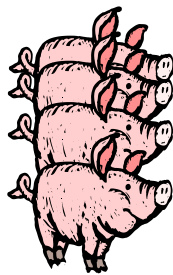
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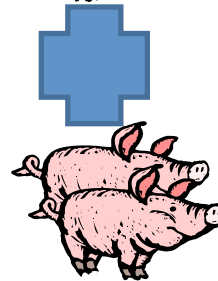
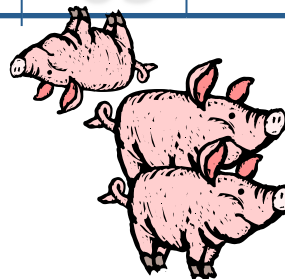
TRANSMISSION DATA OF PIGS RECOVERED FROM LOW VIRULENT ISOLATES

NHV P68

10^5 TCID₅₀
(1ml/pig)dpi



Slaughtered
Studies for virus
presence in tissues



4,5 months
END OF THE
EXPERIMENT

**TWO CONTACT
PIGS
Get infected by ASFV**

- VIRUS TRANSMISSION FROM CARRIER pigs (don't showing clinical signs) TO CONTACT PIGS OCCURED AFTER 2,5 months,
- CARRIERS: VIRUS IN TISSUE TILL 99 DPI.

RECOGNITION OF ASF IN THE FIELD



KEEP UPDATING THE KNOWLEDGE OF CLINICAL PRESENTATIONS



EDUCATION

UPDATE EPIDEMIOLOGICAL INFO
Vet. Authorities,
Veterinarians,
Farmers,
Diagnosticsians,...

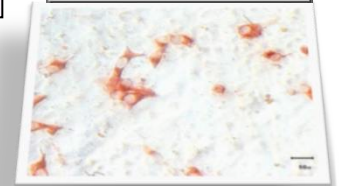
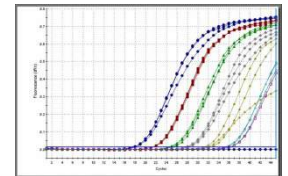
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Laboratory Diagnosis



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CONTROL OF ASF (THOUGH NOT ENOUGH)

AFRICAN SWINE FEVER DIAGNOSIS

INFECTION

DISEASE/DEAD

CARRIERS

Dinamic of ASF Infection

VIRUS

VIRUS AND
ANTIBODIES

ANTIBODIES and
INTERMITTENT VIRAEMIA

DNA/Ag Detection
Techniques

DNA and Antibody Detection
Techniques

0 dpi

7 dpi

35 dpi

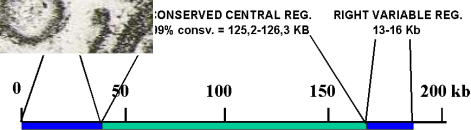
CLINICAL SIGNS

CARRIERS



LABORATORY DIAGNOSIS

Virus detection techniques

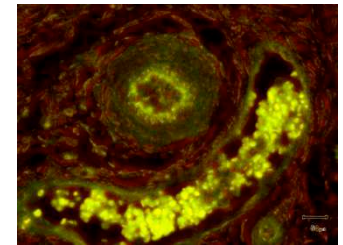


(A) DETECTION OF THE VIRUS GENOME:

PCR

(B) DETECTION OF VIRUS ANTIGENS

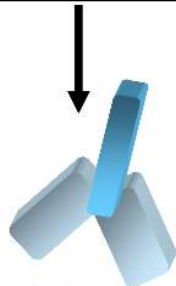
1. Direct immunofluorescent test (DIFT)
2. ELISA for antigen detection



(C) VIRUS ISOLATION AND

IDENTIFICATION BY THE HAEMADSORPTION TEST (HAD)

Antibody detection techniques



Ab detection

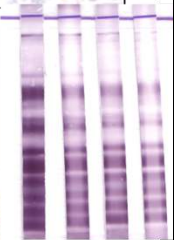
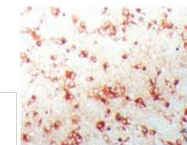
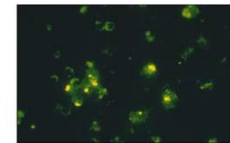
(A) SCREENING BY ELISA

1. OIE-ELISA (Indirect ELISA)
2. COMMERCIAL ELISAs



(B) CONFIRMATORY TESTS

1. Immunoblotting test (IB)
2. Immunoperoxidase test (IPT)
3. Indirect Immunofluorescence test (IFI)



AVAILABLE DIAGNOSTIC TESTS

VIRUS DETECTION TECHNIQUES

AVAILABLE TESTS		TYPE, In house/ Commercial	Recommended Use	REFERENCE
Virus Isolation		*VI /Haemadsorption (HAD) test (i.h.)	Confirmation of primary outbreak.	Malmquist and Hay, 1960
Antigen detection		*Direct Immuno fluorescence (FAT) (i.h.)	Individual testing	Bool et al., 1969
		ELISAIngezim-K2, Double AbSandwich/ Commercial	Surveillance Herd testing	INGENASA
		ELISA (i.h.)	Not in use	Pastor et al.1990; Hutchings and Ferris, 2006;
PCR	Conventional	*Conventional (i.h.)	Surveillance Individual and Herd testing	*Aguero et al. 2003.
		Multiplex ASF-CSF (i.h.)	Co-circulation ASF and CSF	Aguero et al. 2004.
	Real Time	Taqman Probe (i.h.)	Surveillance Individual and herd testing	*King et al., 2003; *Zsack et al. 2005; Tignon et al. 2011
		UPL Probe (i.h.)	Surveillance Individual and herd testing	Fernandez-Pinero et al. 2013
		MGB Probe (i.h.)	Not in use	McKillen et al., 2010
		TETRACORE dried down (Commercial)	Surveillance Individual and herd testing	TETRACORE
		Multiplex ASF-CSF (i.h.)	Co-circulation ASF and CSF	Haines et al.2013
Isothermal Tests		Invader Assay	Not in use	Hjertner et al., 2005
		LAMP assay	Not in use	James et al., 2010

**Included in the OIE Terrestrial Manual for Diagnostic Test and Vaccines, 2012.*

AVAILABLE DIAGNOSTIC TESTS

ANTIBODY DETECTION TECHNIQUES

AVAILABLE TESTS	TYPE, In house/ Commercial	Recommended Use	REFERENCE
ELISA Tests	*OIE Indirect ELISA (i.h.)	Surveillance Herd testing	Sánchez-Vizcaíno et al. 1982; Pastor et al., 1990.
	Recombinant proteins (rp)-ELISA (i.h.)	Surveillance Herd testing	Gallardo et al. 2006,2009, Pérez-Filgueira et al., 2006
	ELISA Ingezim-K3, Bloking/Commercial,	Surveillance Herd testing	INGENASA
	ELISA ID-VET Indirect/Commercial	Surveillance Herd testing	Not available
	ELISA-Svanova Indirect/Commercial	Surveillance Herd testing	Not available
Pen side Tests	Ingezim PPA-CROM Commercial	Surveillance Individual Testing	INGENASA
	Dot Blot (i.h.)	Surveillance Individual Testing	Pastor et al. 1992
Confirmatory Antibody tests	*Immunoblot (IB) Test (i.h.)	Confirmatory Herd testing	Pastor et al. 1989
	*Immunofluorescence Antibody (IFA) test (i.h.)	Confirmatory Herd testing	Pan et al., 1974
	Indirect Immunoperoxidase test (IPT)	Confirmatory Herd testing	Gallardo et al.2013

**Included in the OIE Terrestrial Manual for Diagnostic Test and Vaccines, 2012.*

SUMMARIZING AVAILABLE DIAGNOSTIC TESTS

Antibody Detection Techniques

- ELISA K3*
- ELISA OIE
- ELISA ID-VET*

GOOD SENSITIVE, SPECIFIC AND

RAPID "IN HOUSE" AND

COMMERCIAL TECHNIQUES

- IB TEST
- IPT TEST
- IFA TEST

* COMMERCIAL KITS

Virus Detection Techniques

- Virus Isolation/HAD
- FAT (DIF) TEST

Antigen ELISA K2*

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

guero

PENSIDE TEST FOR ASF ANTIBODY DETECTION , validated and commercially available

INGENASA

INGEZIM PPA CROM

R.11.PPA.K41



INGEZIM PPA CROM es un ensayo enzimático basado en la técnica Inmunocromatografía Directa, que utiliza un anticuerpo monoclonal (AcM) específico de la proteína Vp72 virus de la Peste Porcina Africana.

SUERO POSITIVO **SUERO NEGATIVO**



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/rosa. 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 porcino.

SENSIBILIDAD DIAGNÓSTICA

- 84 sueros de feroqueros 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

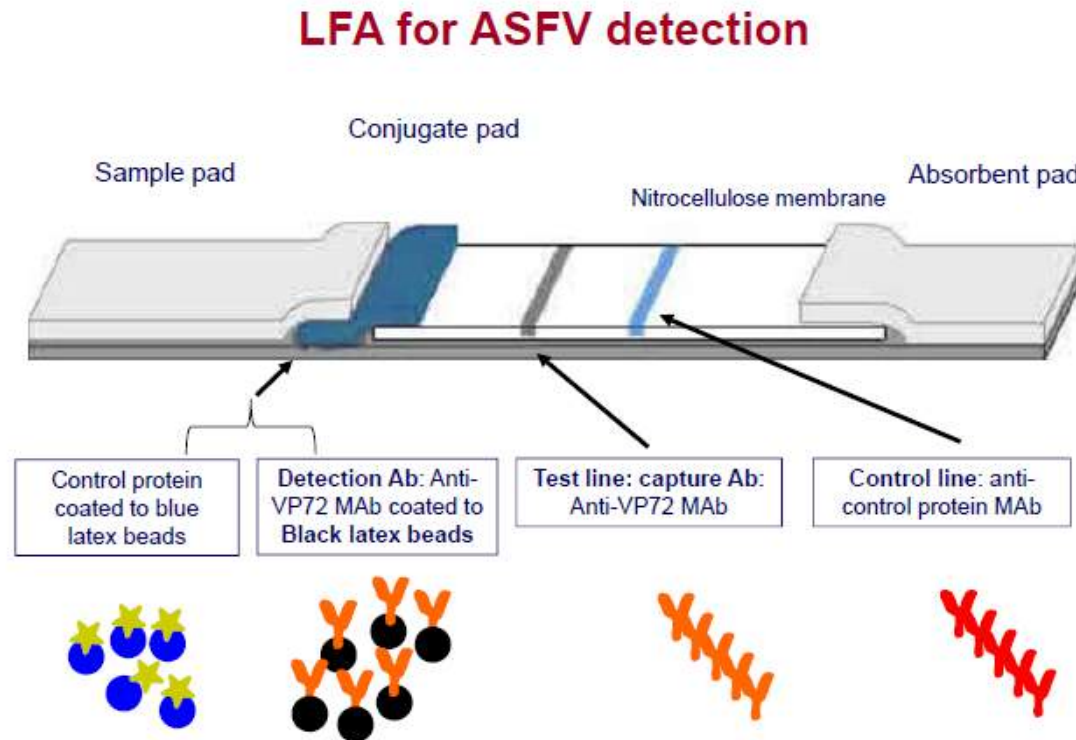
- Suero de 13 cerdos experimentalmente infectados con diferentes aislados del virus de la PPA.
- Sueros de Referencia de la OIE fuertemente positivo y débilmente positivo.

El ensayo es capaz de detectar anticuerpos entre los días 10 p.i. y 21p.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.

PENSIDE TEST FOR ANTIGEN DETECTION developed and initial validation performed

INGENIASA



ADDITIONAL FIELD VALIDATION IS ON-GOING



ASF EPIDEMIC IN THE EU COUNTRIES, 2014 SOME FINDINGS CONCERNING DIAGNOSIS

Overall analysis of the results: More than 240 cases and outbreaks by ASF circulating virus induce Acute infection.

W **A PERCENTAGE OF ANIMALS SURVIVES ONE MONTH** **by**
IPT **AFTER INFECTION** **he**

Domestic pigs; The presence of antibodies was confirmed by IPT test in 46.8% domestic pig.



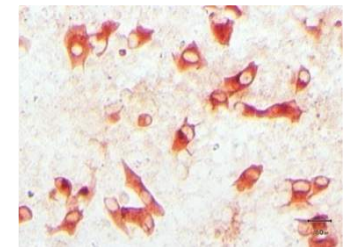
ASF EPIDEMIC IN THE EU COUNTRIES, 2014

SOME FINDINGS CONCERNING DIAGNOSTIC TECHNIQUES

- 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 .

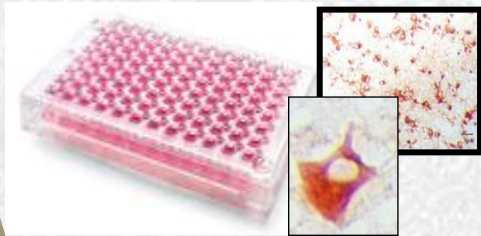




DIAGNOSIS

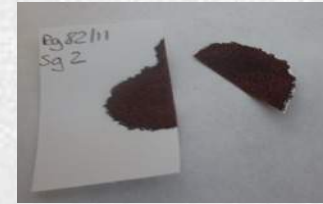
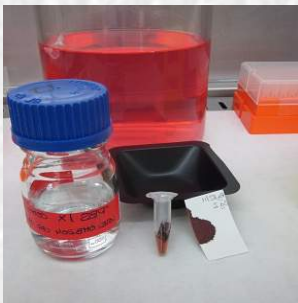
what is now coming.....

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 a 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

Å. Utenthal^{a,*}, U.C. Braae^b, H.A. Ngowi^c, T.B. Rasmussen^a, J. Nielsen^a, M.V. Johansen^b

^aSection for Virology, National Veterinary Institute, Technical University of Denmark, Lindholm, DK-4771 Kalvehave, Denmark

^bSection for Parasitology, Health and Development, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, DK-1870 Frederiksberg, Denmark

^cDepartment of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania

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.



The background of the slide is a light gray, semi-transparent image of a hand holding a pen, poised to write on a document. The image is centered and occupies the entire background. The text is overlaid on a white rectangular area in the center of the slide.

**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.

NEED FOR MORE FIELD SAMPLES FROM AFRICA REGIONS

- 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**

DIAGNOSIS: RESEARCH IN PROGRESS



- **Defining New genetic markers** to trace the source of the outbreaks and so the dynamic of the disease

NEED FOR MORE FIELD SAMPLES FROM AFRICA REGIONS

- **Deeper full genome sequence of currently circulating isolates in Europe** is being performing 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.

DIAGNOSIS: FUTURE PRIORITIES



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.

DIAGNOSIS: FUTURE PRIORITIES



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.



FROM OUR EXPERIENCE , WE HAVE LEARNED:

EPIDEMIOLOGY



LABORATORY
DIAGNOSIS

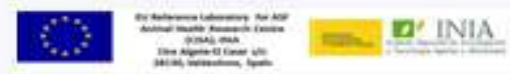
A GOOD DIAGNOSIS OF ASF IS ESSENTIAL FOR THE CONTROL OF ASF , BUT NOT ENOUGH, INFORMATION for implementing GOOD ASF CONTROL ESTRATEGIES is needed.



ANNUAL MEETINGS - THE DISEASE - **EURL FOR ASF** - ASF DIAGNOSIS - DIAGNOSTIC SERVICES - SEQUENCE DATA BASE - LINKS

WELCOME TO THE EUROPEAN UNION REFERENCE LABORATORY FOR AFRICAN SWINE FEVER (EURL-ASF).

CENTRO DE INVESTIGACION EN SANIDAD ANIMAL (CISA-INIA)



eurl.asf@inia.es

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

NEW! THREAT OF ASF SPREAD IN EASTERN EUROPE: URGENT NEED FOR INTERNATIONAL COLLABORATION. TECHNICAL MEETING 4-5 DECEMBER 2012, BUDAPEST, HUNGARY

WORKSHOP ON LABORATORY DIAGNOSIS OF ASF and CSF May 30-June 1 2012. Hannover, Germany.

<http://asf-referencelab.info>

IT IS THE TIME TO BE READY

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2007-2015



ARE YOU READY?



THANK YOU FOR YOUR ATENTION

Thanks to the
AFRICAN and
EUROPEAN
TEAMS working
with us in ASF and
to the



THANK YOU CADMS
THANK YOU UC DAVIS



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