Státní veterinární ústav Jihlava

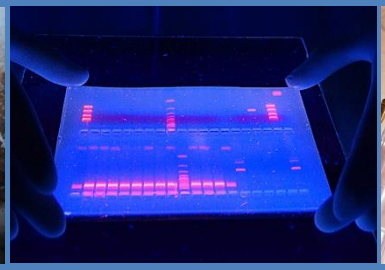
ASFV laboratory diagnostics and capability in the Czech Republic

MVDr. Petr Václavek, Ph.D.

NRL for ASF, State Veterinary Institute Jihlava

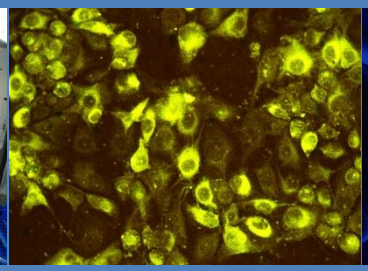
SGE ASF8, Chisinau, Moldova, 20-21 September 2107

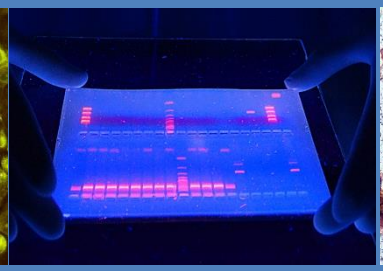


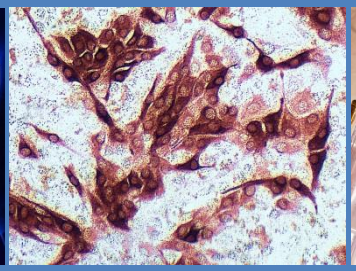




STATE VETERINARY INSTITUTE JIHLAVA

- **veterinary laboratory** - services in the areas of **animal health, food safety and ecology**
- semi-budgetary organization established by the Ministry of Agriculture and incorporated into the organizational structure of the State Veterinary Administration
- two Reference Laboratories and seven NRLs included **NRL for ASF**







Close cooperation with EURL

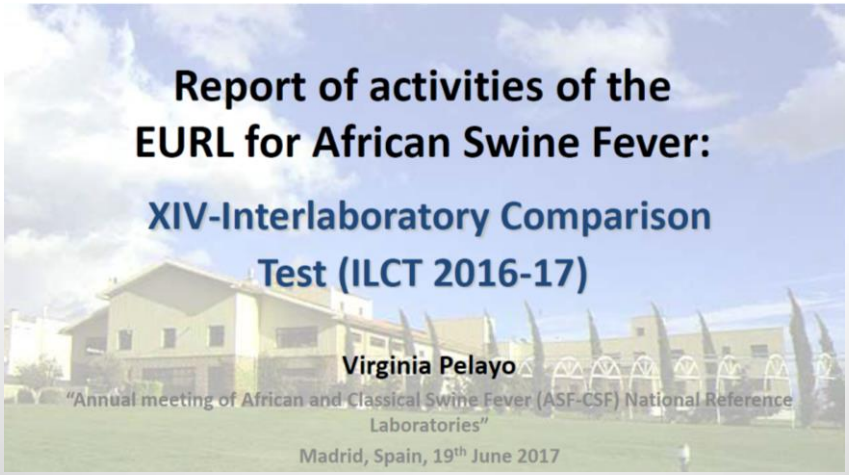
Interlaboratory Comparison Tests

organized by
European Union reference laboratory for ASF
Centro de Investigación en Sanidad Animal (CISA-INIA)
CISA-INIA. Valdeolmos, Madrid, SPAIN

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

MINISTERIO
DE ECONOMÍA, INDUSTRIA
Y COMPETITIVIDAD

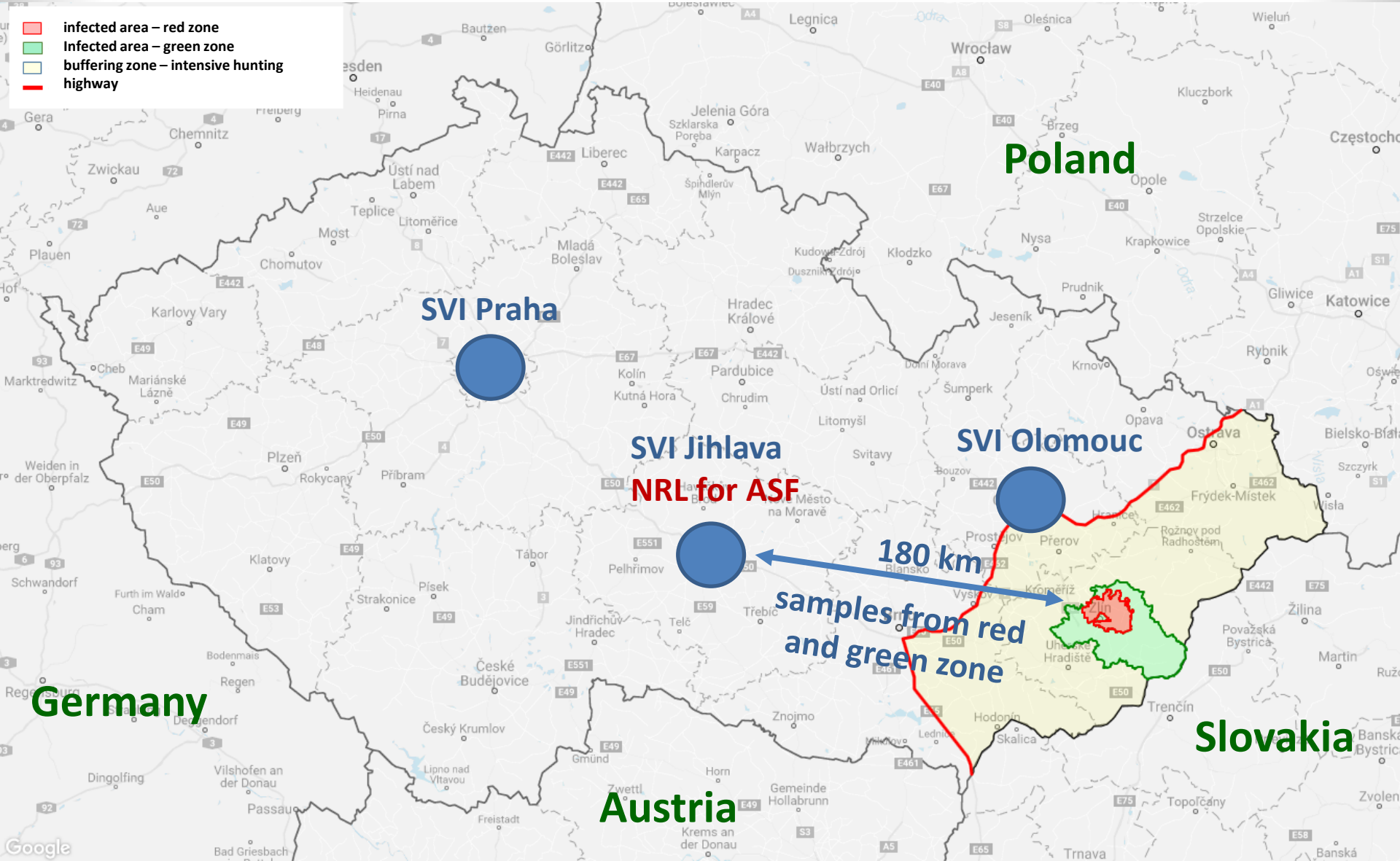
INIA
Instituto Nacional de Investigación
y Tecnología Agraria y Alimentaria



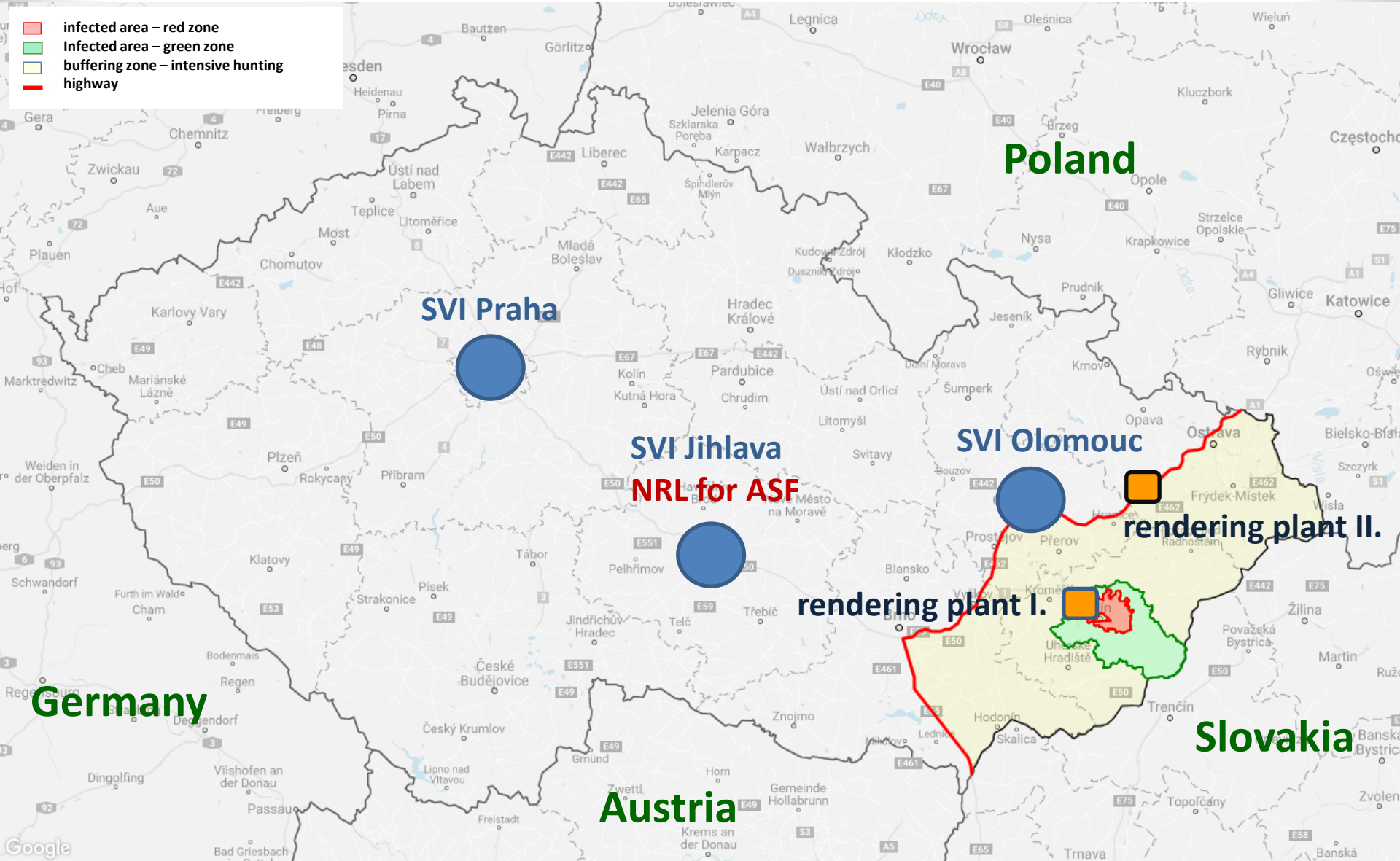
Czech Interlaboratory Comparison Tests

organized by
Czech National Reference laboratory for ASF
State Veterinary institute Jihlava

SVI laboratories in the Czech Republic



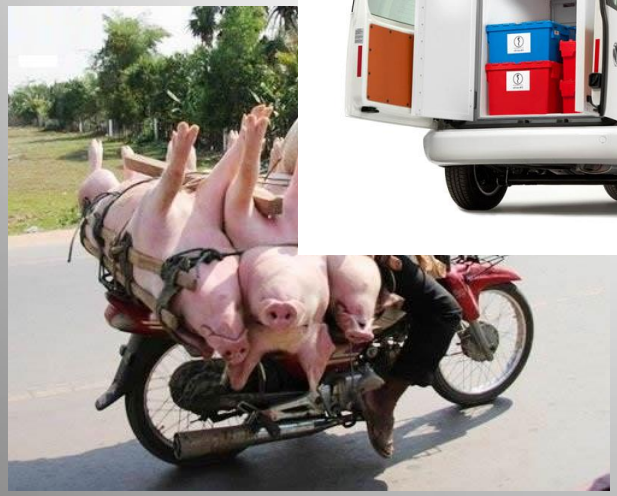
SVI laboratories in the Czech Republic



First ASF outbreak in country =

challenging BUT time-consuming and exhausting time for laboratories

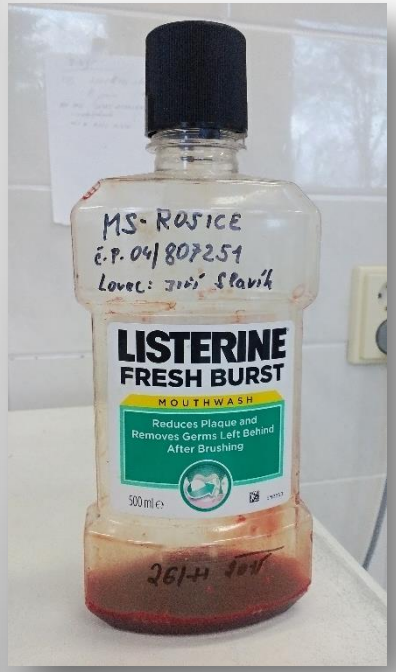
everyday transport of samples + everyday afternoon testing

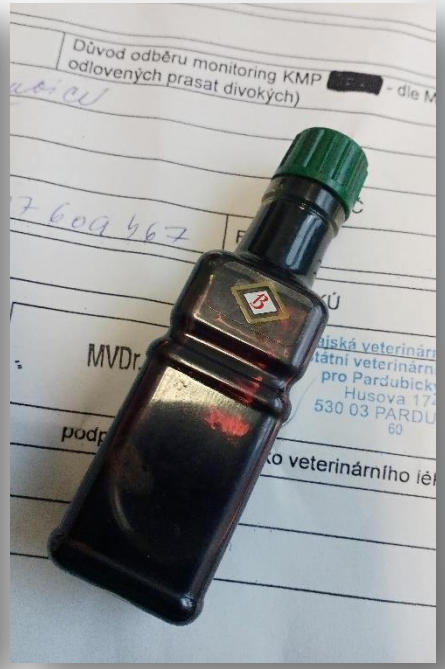


Sampling: sample types and quality

1) **blood** – first choice sample
2) **spleen**
3) **bone marrow**
4) **kidney, lung, tonsils etc.**

Alternative ways how to collect blood samples for CSF and ASF





Sample logistic and pretreatment

- **preparation** of tissue samples suspension and blood sample purification are necessary BUT **time consuming procedures**

- **acceleration of the proces:** e.g. homogenisation of tissue samples (10% wt/vol) – speed-up due the grinding homogenisator Omni Bead Ruptor (24 samples per 2min.)

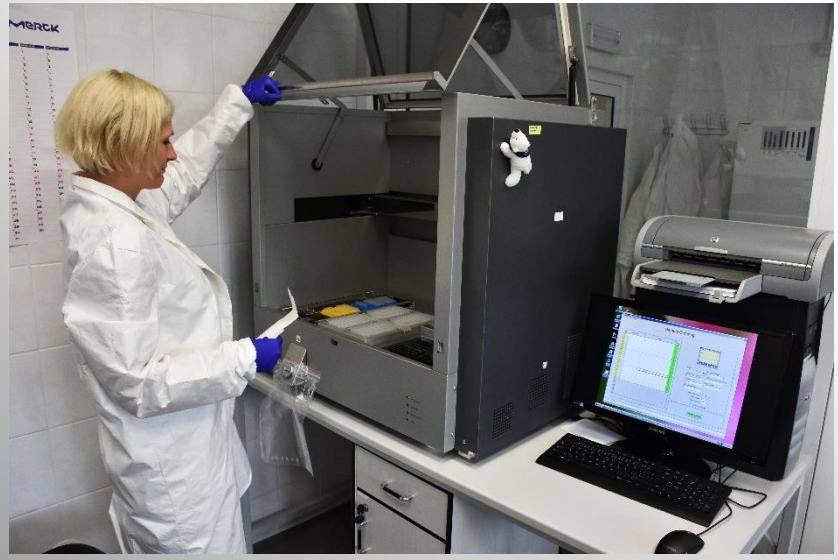
Laboratory capacity - PCR

The ability to integrate qPCR into **automated platforms** increases sample throughput and decreases the potential for cross-contamination.

- fast, sensitive, quantitative, closed system, 96-wel format
- e.g. enhancement of lab capacity due the **robotic extraction** of DNA:

❖ MagNA PURE (Roche) – 32 samples/1 run

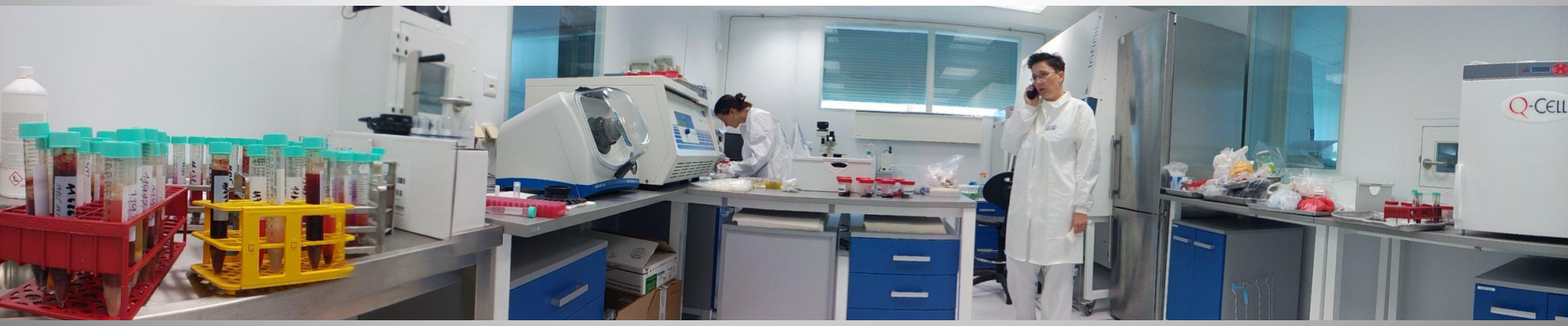
❖ QIA Symphony (Qiagen) – 96 samples/1 run



Approximate capacity of Czech NRL for ASF

(samples tested/1day)

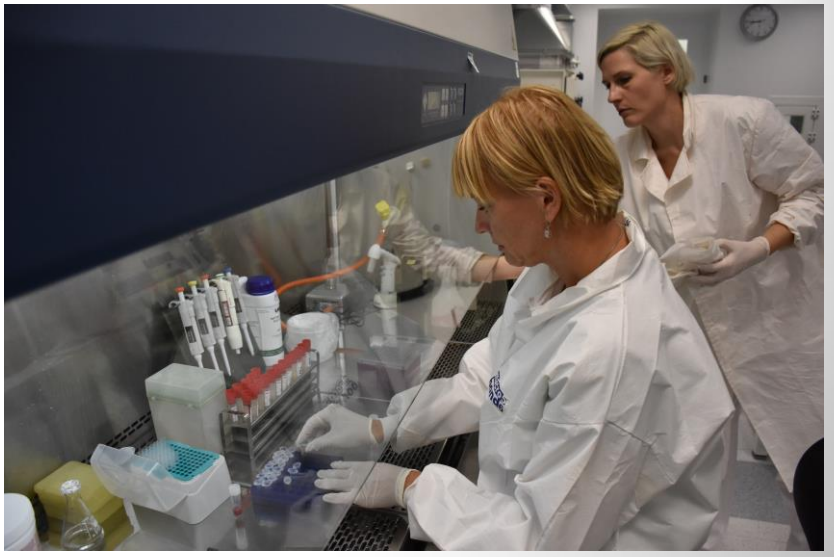
	Real Time PCR		ELISA Ab
	blood	tissue	blood
standard mode	500	300	2000
crisis mode	1000	500	4000



Approximate total capacity of all Czech SVI laboratories

(samples tested/1day)

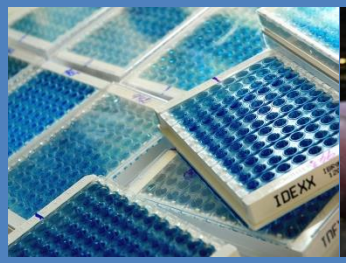
	Real Time PCR		ELISA Ab
	blood	tissue	blood
standard mode	1200	650	5000
crisis mode	1900	1000	10 000



DIAGNOSTICS TESTS used in CZECH LABORATORIES

ANTIBODY DETECTION TECHNIQUES

TEST	TYPE	Recommended use	REFERENCE
ELISA test	**INGEZIM PPA Compac R.11.PPA.K3 blocking ELISA** – based on monoclonal antibody (MAb) specific to ASFV VP72 protein	surveillance herd testing	INGENASA
	ID Screen Indirect ELISA - based on 3 recombinant antigens : P32, P62, and P72	surveillance herd testing	ID.VET
	ID Screen Competition ELISA – based on p32 recombinant protein	surveillance herd testing	ID.VET
	SVANOVIR® ASFV-Ab indirect ELISA - based on the recombinant p30 protein	surveillance herd testing	Svanova



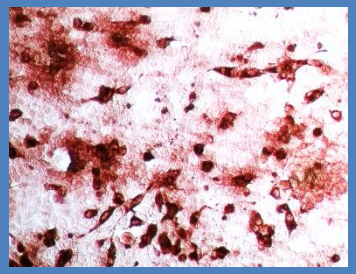


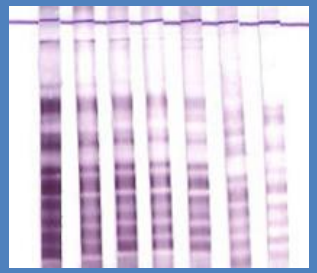

DIAGNOSTICS TESTS used in CZECH LABORATORIES

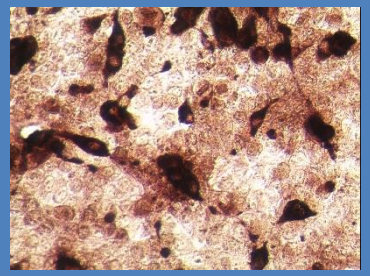
ANTIBODY DETECTION TECHNIQUES

Confirmatory Antibody Tests

TEST	TYPE	Recommended use	REFERENCE
IB test	Immunoblot (IB) test	**confirmatory test** (only NRL lab)	Pator et al. 1989
IPT test	Indirect immunoperoxidase test (IPT)	**confirmatory test** (only NRL lab)	Gallardo et al. 2013



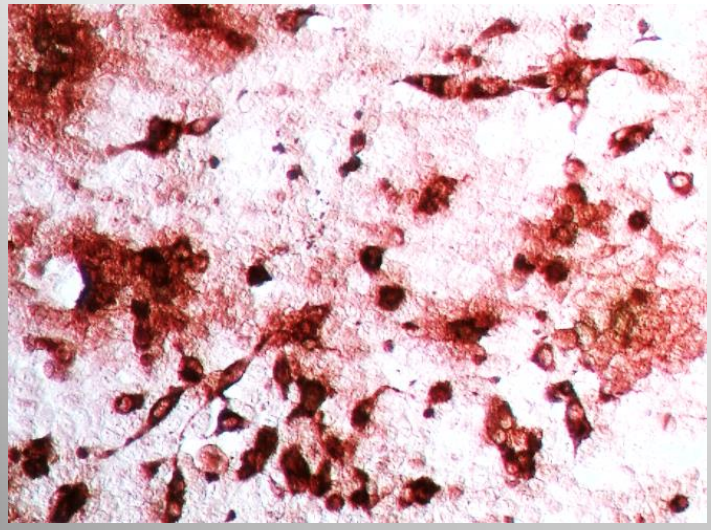


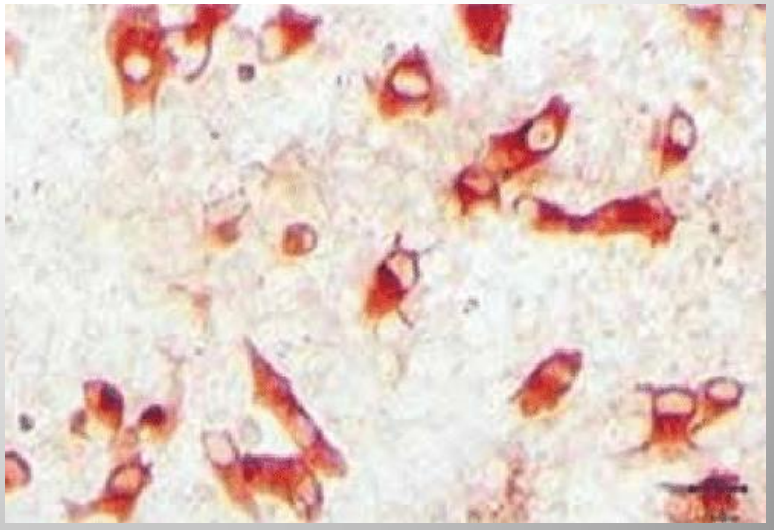


Indirect immunoperoxidase test (IPT)

- Spanish isolate ASFV Ba71VR adapted on VERO cells
- specific antibody detection on kidney monkey cells infected with adapted virus
- infected cells fixed (coated 96-well plates)
- **confirmatory technique** for positive and doubt ELISA results
- samples: sera, exudate tissue, dried blood filter paper sample

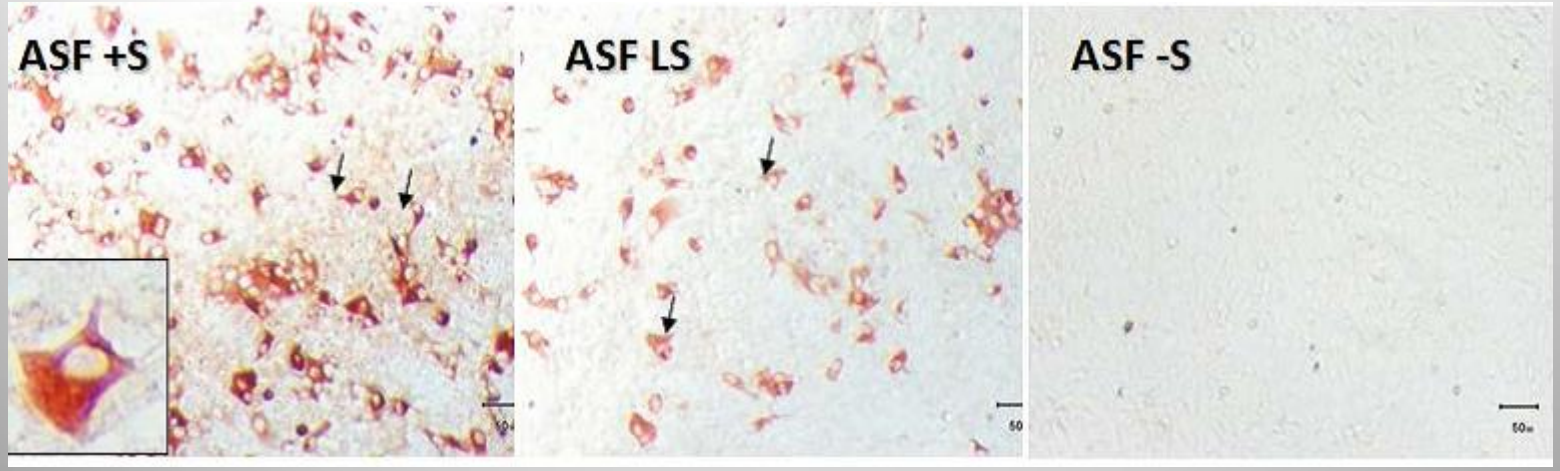
high sensitivity (98,2%) and specificity (98,95%)





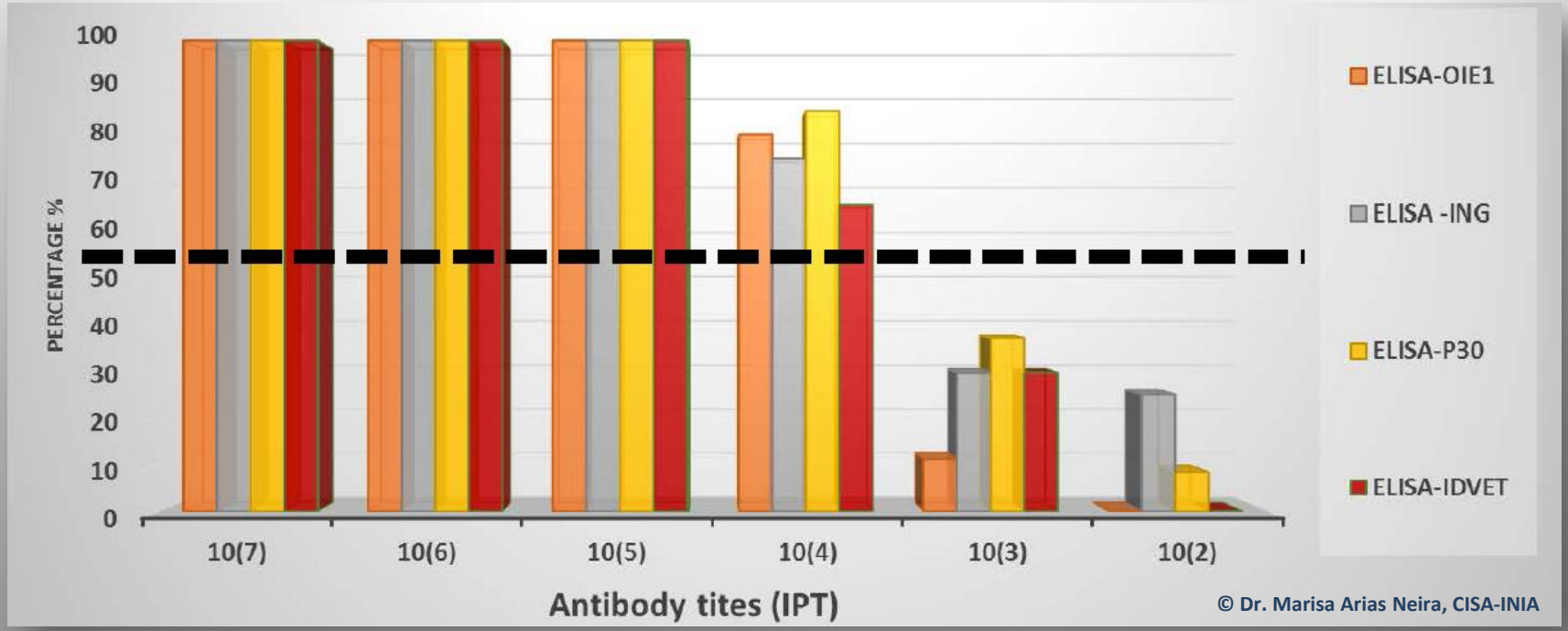
Indirect immunoperoxidase test (IPT)

- IPT is the **best test given its superior sensitivity**
- able to detect antibodies at an earlier point in the serological response (acute, subacute infection)
- more sensitive also in subclinical infection
- availability to test blood, serum and/or exudate tissue samples (7-11 dpi)
- **labour-intensive method, so it cannot be used as screening test**



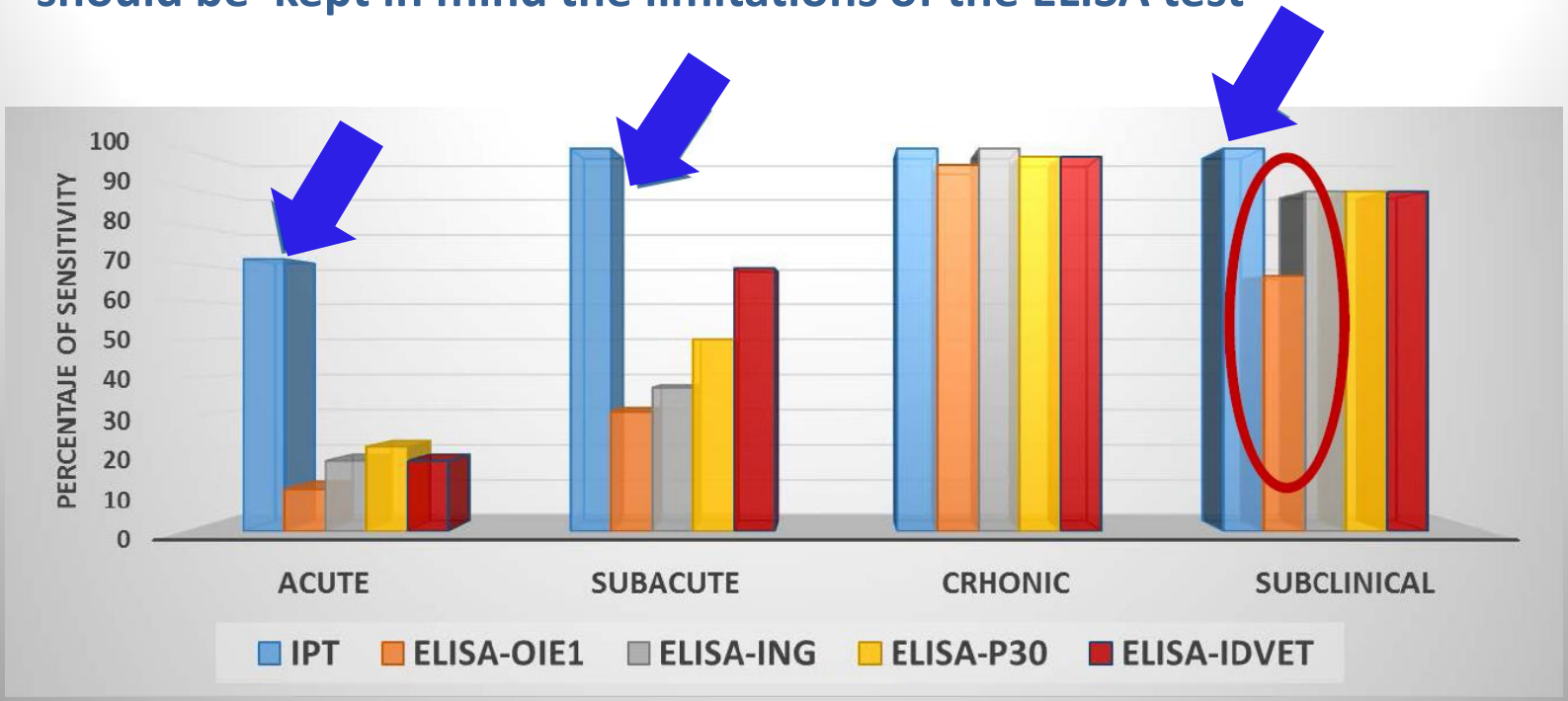
Sensitivity and specificity of serology

The percentage of sensitivity of ELISA decreases when sera with IPT titres < 10(5) are tested



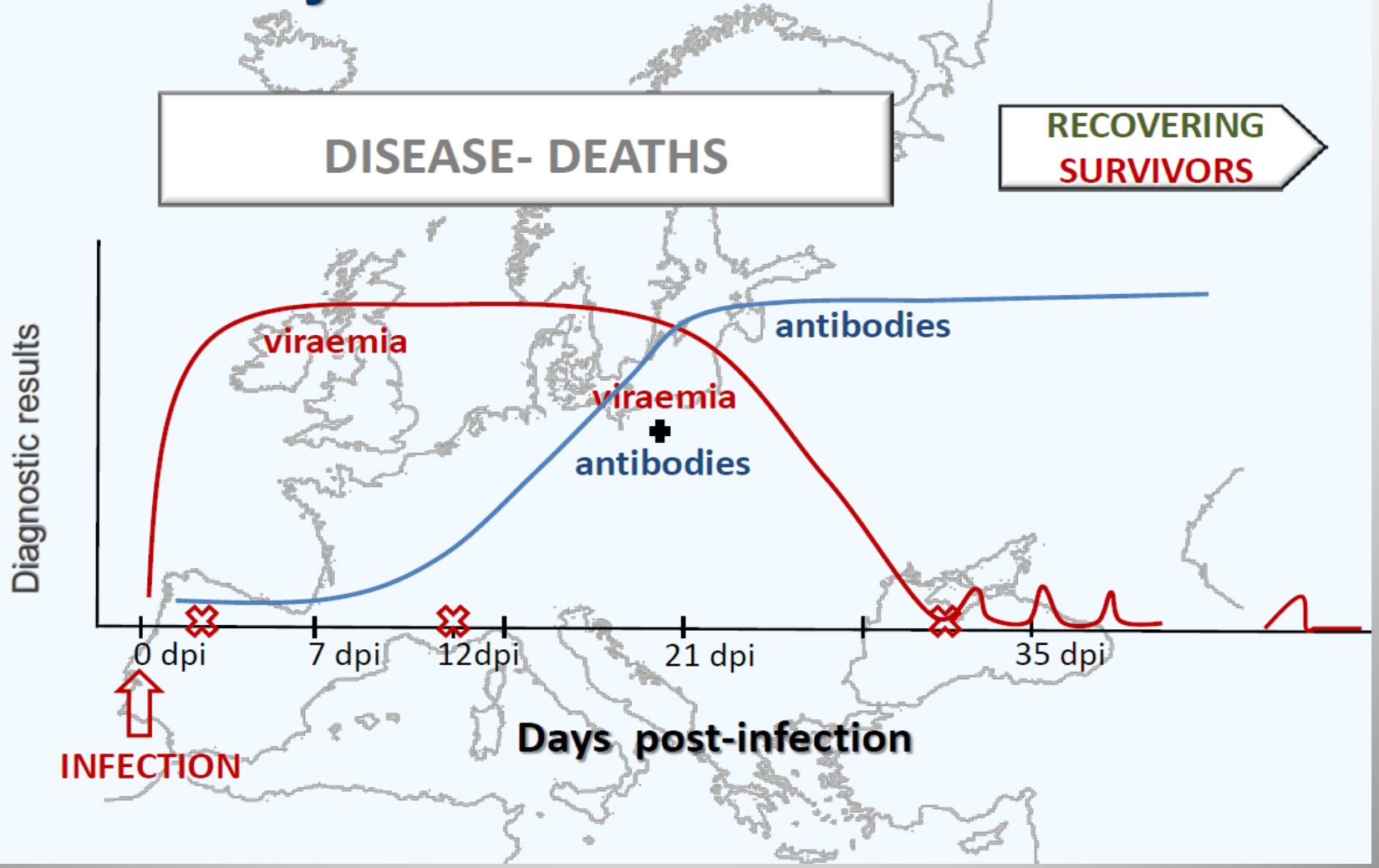
Assesment of test for serology

- **IPT**: highest sensitivity of confirmatory tests
- **ELISAs**: variable sensitivity for subacute forms (depending the time of sampling) and HIGH SENSITIVITY for survivors and recovered from infection and chronic and subclinical forms
- **ELISA** - the most commonly used test to scree for ASF Ab, however it should be kept in mind the limitations of the ELISA test



EPIDEMIOLOGY INVESTIGATION

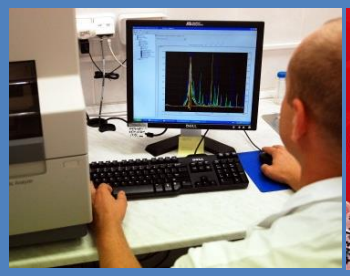
Dynamic of ASFV infection

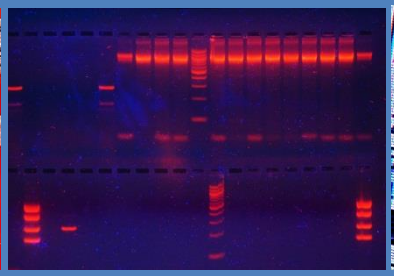


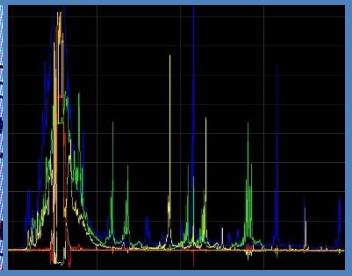
DIAGNOSTICS TESTS used in CZECH LABORATORIES

DETECTION of the ASF VIRUS GENOME by PCR

TEST	TYPE	Recommended use	REFERENCE
Conventional PCR	OIE conventional PCR	surveillance Individual and herd testing	Agüero et al. 2003
Real Time PCR	UPL Real-time PCR (UPL Probe)	surveillance Individual and herd testing	Fernandez et al. 2013
	Taqman Probe (OIE - Real Time PCR)	surveillance Individual and herd testing	King et al. 2003 Zsak et al. 2005
Genotyping (genotype I. and II.)	PCR amplification using primers CRV1/2	confirmatory test (only NRL lab)	Gallardo et al. 2011

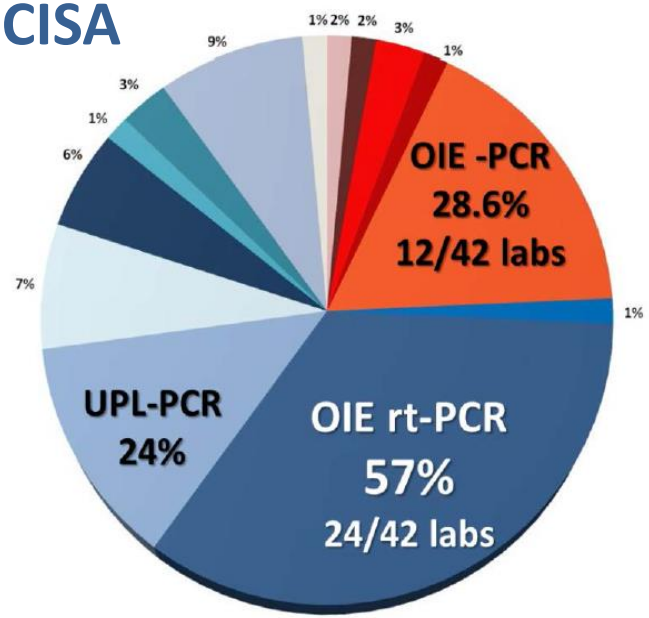




Sensitivity and specificity of PCR tests

Study based on >2500 field and experimental samples (genotype II.) – EURL INIA-CISA



Sensitivity

UPL-PCR > OIE Real Time PCR > OIE-PCR

Specificity

UPL-PCR = OIE Real Time PCR > OIE-PCR

UPL - Real-time PCR (Fernandez-Pinero et al. 2013)

Transboundary and Emerging Diseases

Transboundary and Emerging Diseases

ORIGINAL ARTICLE

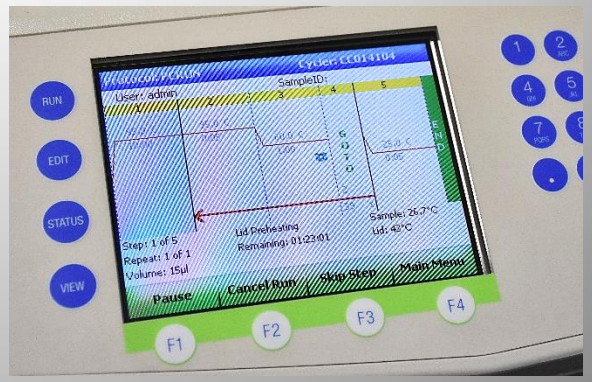
Molecular Diagnosis of African Swine Fever by a New Real-Time PCR Using Universal Probe Library

J. Fernández-Pinero[1], C. Gallardo[1], M. Elizalde[1], A. Robles[1], C. Gómez[1], R. Bishop[2], L. Heath[3], E. Couacy-Hymann[4], F. O. Fasina[5], V. Pelayo[1], A. Soler[1] and M. Arias[1]

[1] Centro de Investigación en Sanidad Animal (CISA-INIA), Madrid, Spain
[2] International Livestock Research Institute (ILRI), Nairobi, Kenya
[3] ARC-Onderstepoort Veterinary Institute, Transboundary Animal Diseases Programme, Pretoria, South Africa
[4] LANADA-LCPA Laboratoire de Virologie, Rue du Jardin Botanique BP 206, Bingerville, Ivory Coast
[5] Department of Production Animal Studies, University of Pretoria and National Veterinary Research Institute, Vom, Nigeria

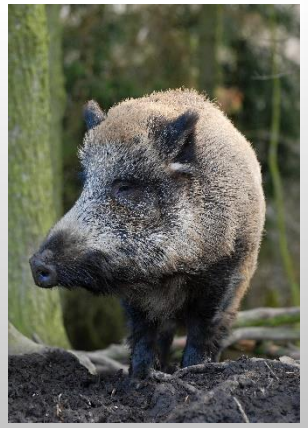
- Real- time PCR using commercial probe (Universal ProbeLibrary no. 162; Roche Applied Science, Branford, CT, USA), which generates an amplicon of 74 bp within viral protein 72, to confirm the presence of ASFV DNA
- combined with specifically designed primer set
- LightCycler 480 Probes Master Kit (Roche Applied Science)
- CFX96 Real-Time System - PCR thermocycler (Biorad)

- **high analytical sensitivity** – able to detect about 18 copies
- **greater diagnostic sensitivity for detecting survivors and allow earlier detection of the disease** (compared to real-time and conventional OIE PCR)



Value and importance of serology

- **surveilance programs: Ab detection is crucial for the detection of survivors/recovered animals** and to determine the time of infection
- a **certain proportion of animals are surviving** the infection
- **ASF diagnosis requires the identification of animals that are or have previously been infected with ASFV**.
- **no vaccine is available against ASFV,** which means that the **presence of anti-ASFV antibodies always indicates infection**.
- **anti-ASFV antibodies appear soon after infection (7-8 dpi) and persist for up to several months or even years.**
- **therefore, the search for antibodies from hunted or dead animals is essential for obtaining a complete picture of the epidemiology in question at the time of these epidemic outbreaks, and for determining the date of the infection.**

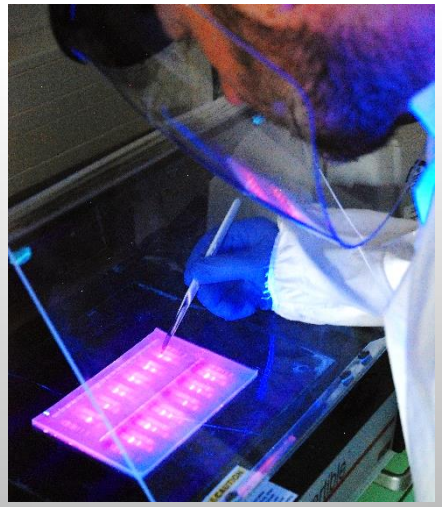



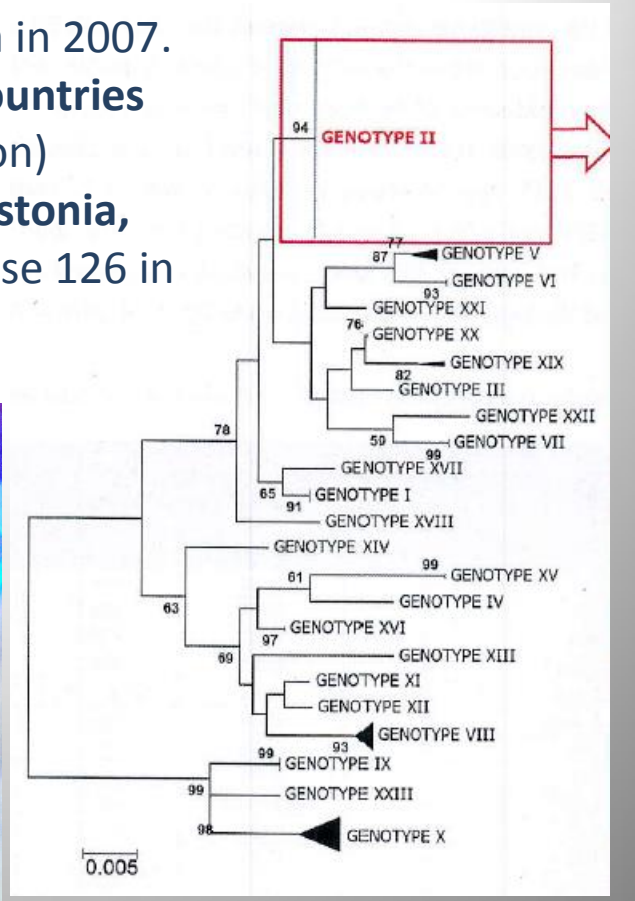

Molecular characterisation of Czech ASF strain in EURL for ASF

(INIA-CISA, Valdeolmos, Madrid, Spain)

The p72 genotyping of the Czech Republic wild boar ASFV strain clustered the virus within **p72 genotype II** circulating in the Eastern European countries since the first introduction in Georgia in 2007.

- **subtyping** = **identical to ASFV circulating in Eastern countries since 2007 except certain areas of Estonia** (Tartu region)
- **100% homologous with viruses in Lithuania, Latvia, Estonia, Poland and Russia Federation** (except the virus WB case 126 in Poland 2016)





Molecular characterisation of Czech ASF strain in EURL for ASF

(INIA-CISA, Valdeolmos, Madrid, Spain)

Table 4 → Summary of the molecular characterization results from Eastern European ASFV isolates 2007-2017

COUNTRY	YEAR	P72 GENOTYPE	CVR SUBTYPING	$IGR_{I73R-I329L}$ SUBTYPING	IGR_{MGF} SUBTYPING
Georgia	2007	II	CVR1	IGR-1	MGF -1
Armenia	2007	II	CVR1	IGR-1	MGF -1
Azerbaijan	2008	II	CVR1	IGR-1	MGF -1
Russia Federation	2007-2012	II	CVR1	IGR-1	MGF -1
	2012-2016	II	CVR1	IGR-1 + IGR-2	MGF -1 + MGF -2
Ukraine	2012, 2015	II	CVR1	IGR-2	MGF -1
Belarus	2013	II	CVR1	IGR -2	MGF -1
Estonia	2014	II	CVR1	IGR-2	MGF -1
	2015-2017	II	CVR1 + CVR 2	IGR-2	MGF -1
Latvia	2014-2017	II	CVR1	IGR-2	MGF -1
Lithuania	2014-2017	II	CVR1	IGR-2	MGF -1
Poland	2014-2015	II	CVR1	IGR-2	MGF -1
	2016	II	CVR1	IGR-2	MGF -1 + MGF -2
	2017	II	CVR1	IGR-2	MGF -1
Moldova	2016	II	CVR1	IGR-2	MGF -1
Czech Republic	2017	II	CVR1	IGR-2	MGF -1

Conclusions

Questions?

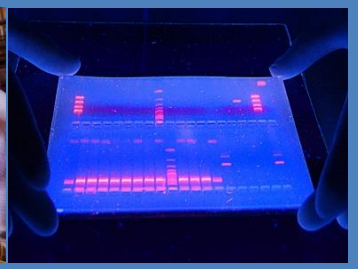
E.g. information campaigns focused on hunters and public:

- increase hunters awareness
- sampling methods + sampling material
- safe removal of pigs found dead or shot
- biosecurity measures during hunting and sampling
- instructions - reporting the finding of each dead WB pig to a competent authority
- Etc.

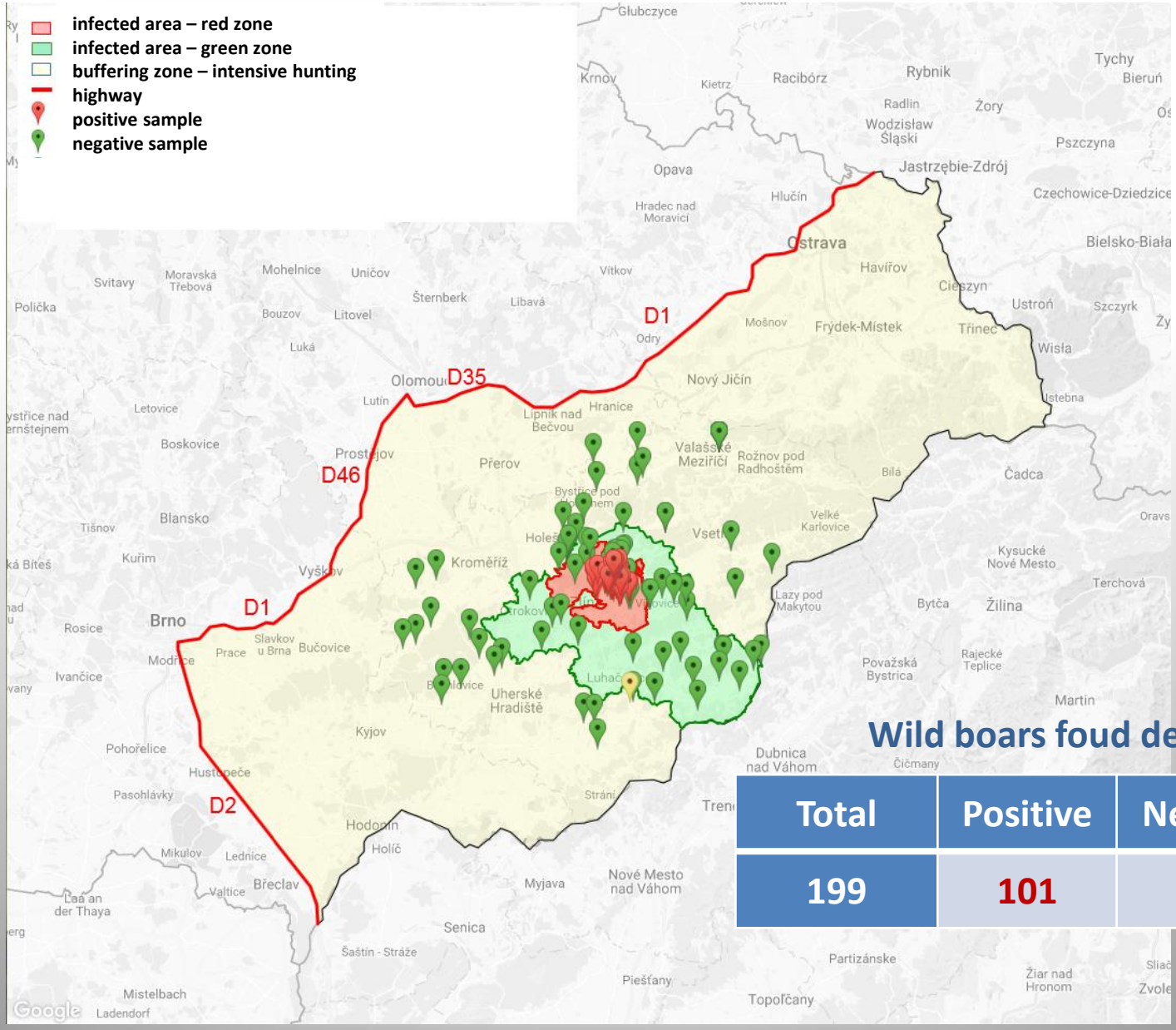
Preparedness! Be ready!!







Active surveillance: wild boars foud dead



Wild boars foud dead

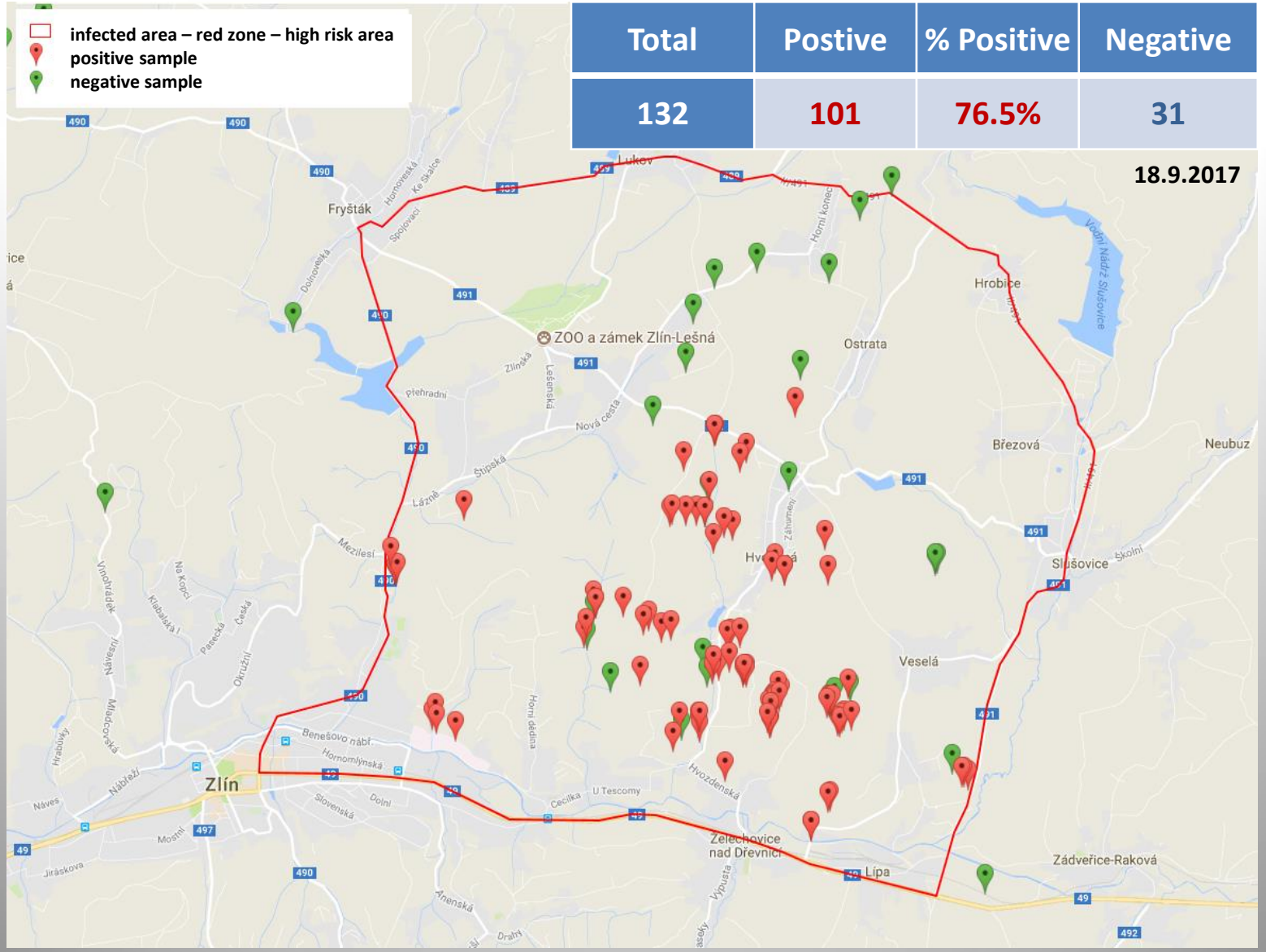
Total	Positive	Negative
199	101	98

18.9.2017

Active surveillance: wild boars foud dead

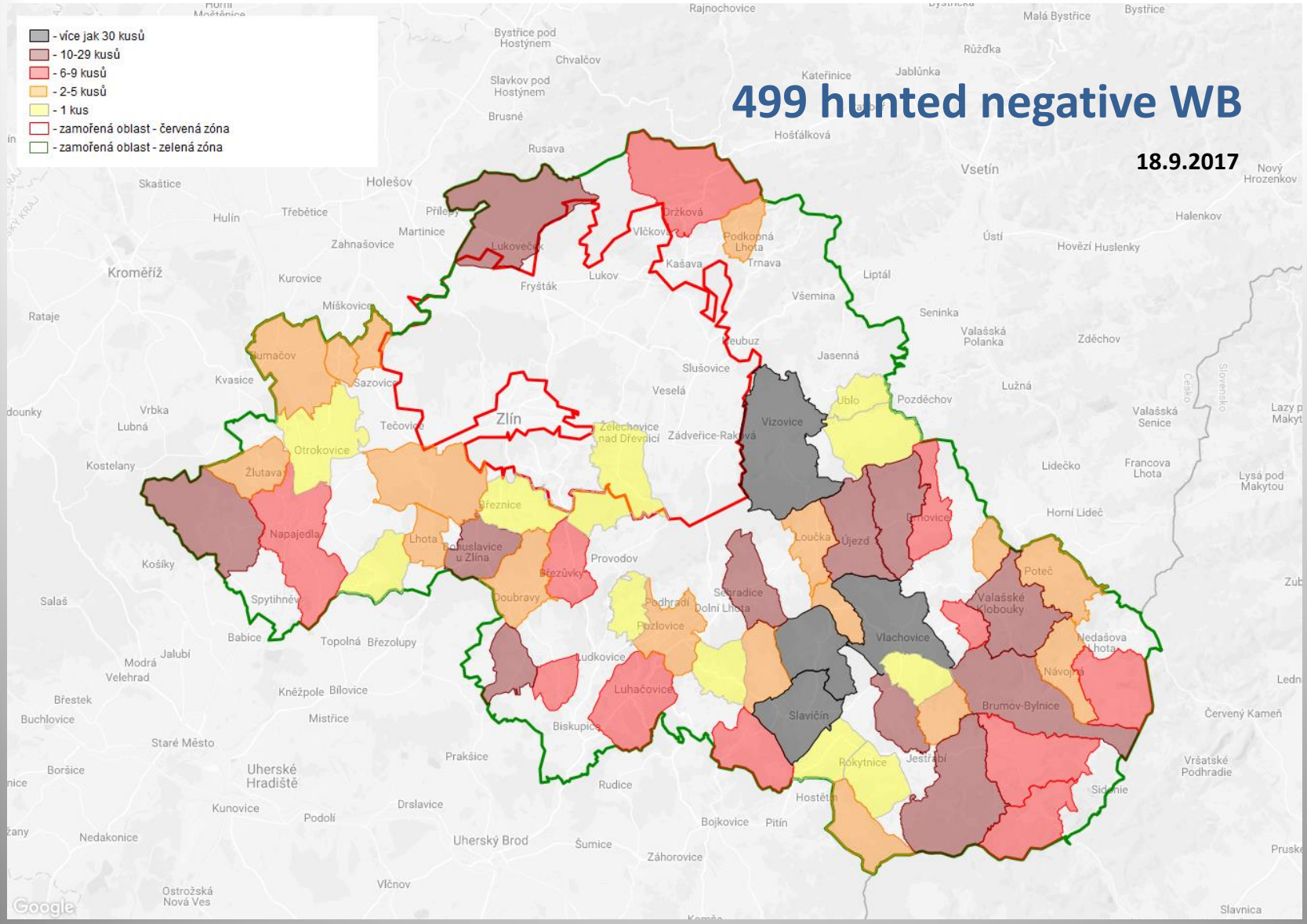
Positive and negative cases of found dead wild boars inside highly risk area inside infected area (inside electric fence)

Total	Postive	% Positive	Negative
132	101	76.5%	31

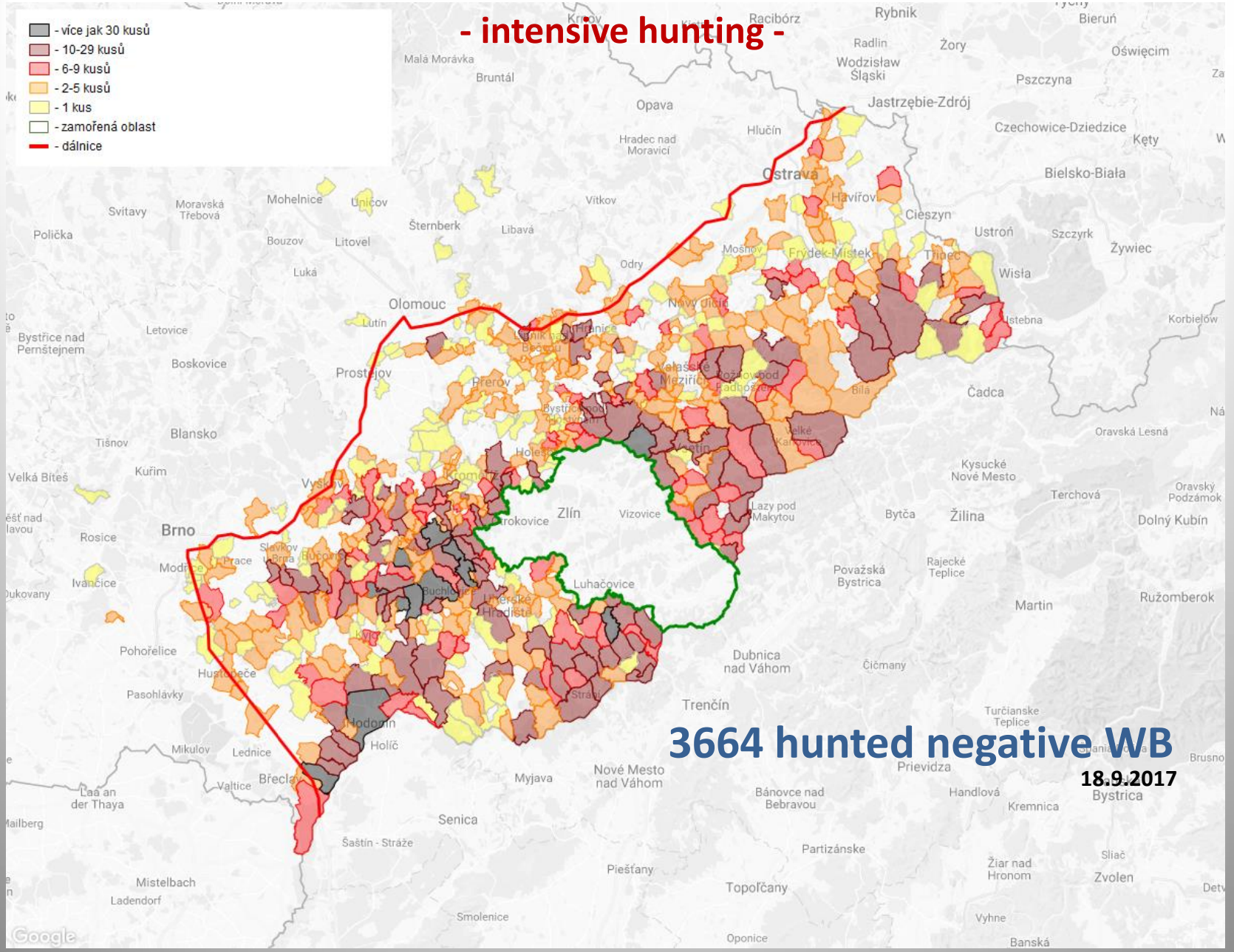


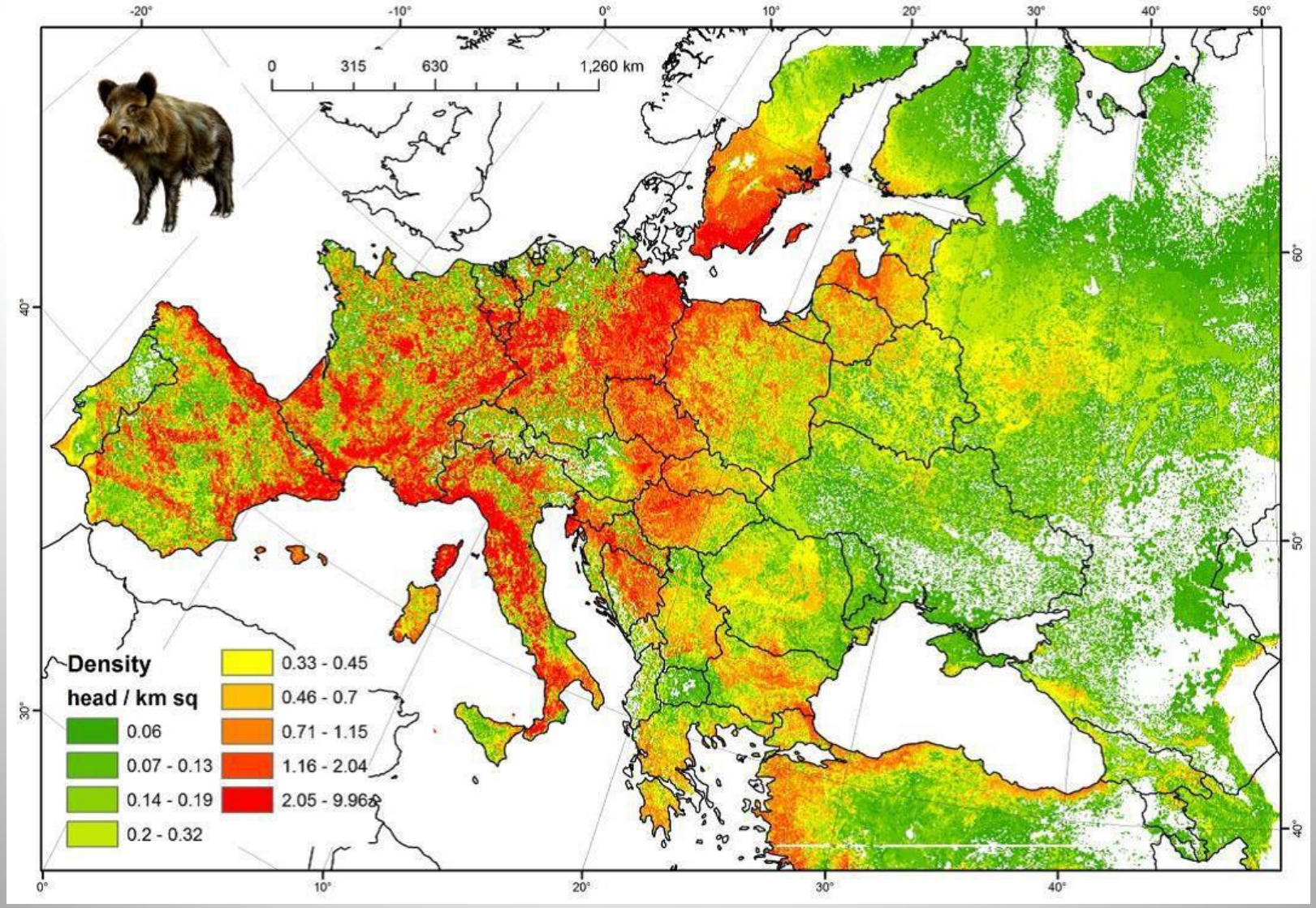
Active surveillance: hunted WB in infected zone

(except red zone)



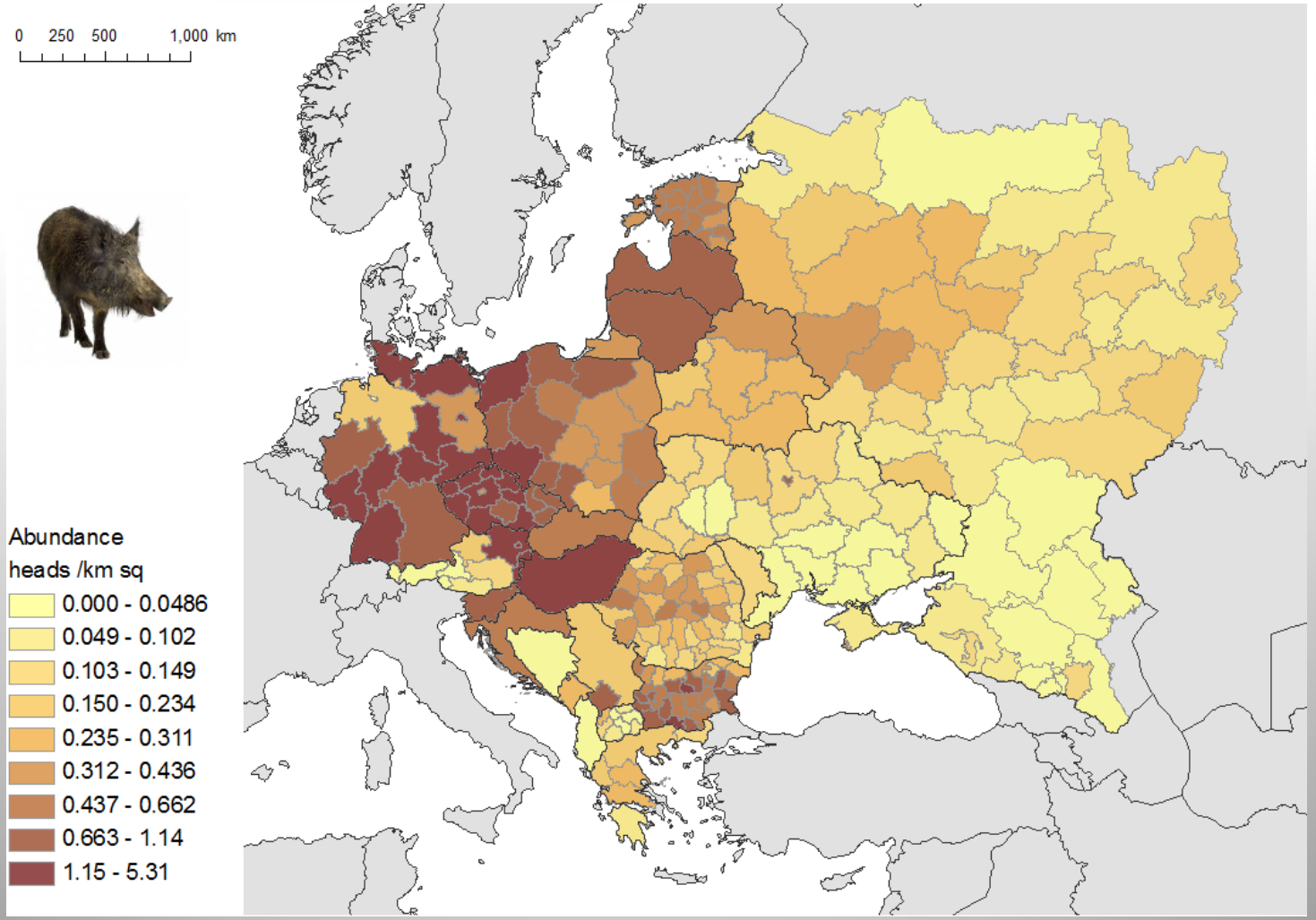
Active surveillance: hunted WB in buffering zone

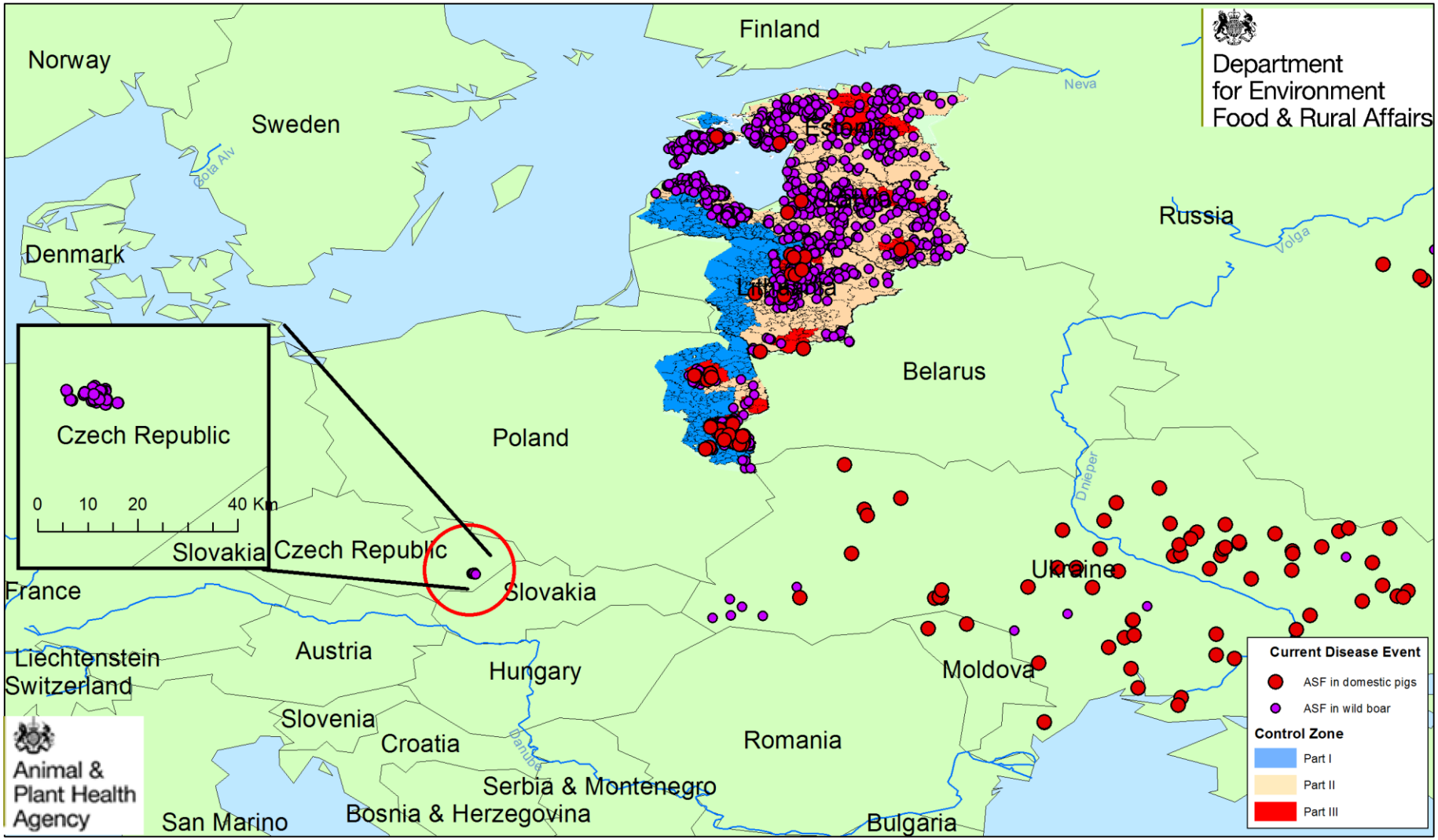




Modelled wild boar population density in Europe (source: FAO/ASFORCE, May 2015)

Wild boar population abundance (head per km^2) based on available population estimates - based on the national wildlife statistics (EFSA Journal 2015;13(7):4163).





Actual Scale 1:11,800,000

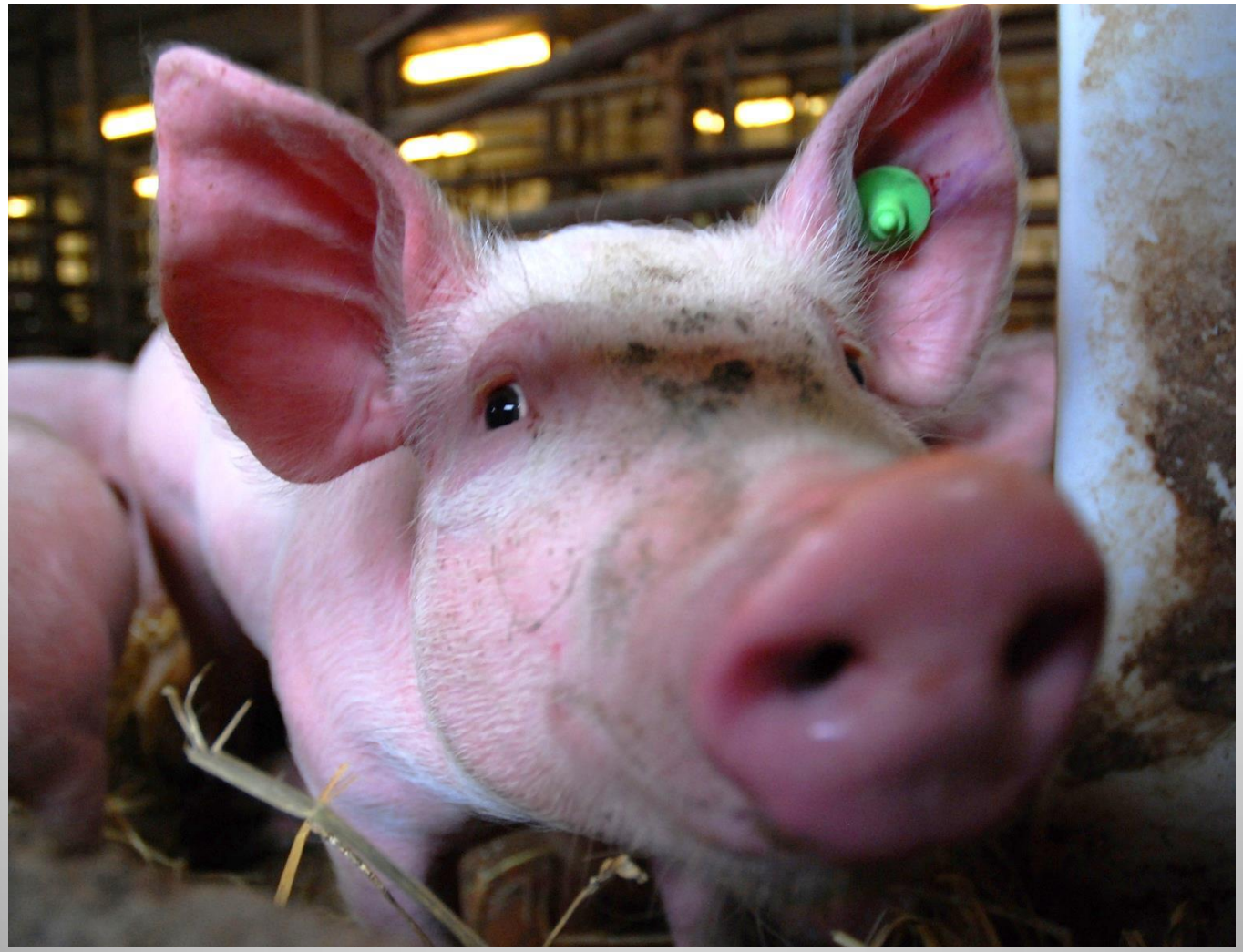
Map prepared by IDM

Recent African Swine Fever outbreaks domestic pigs and wild boar in 2017 [Inset: wild boar cases in Czech Republic]

Date prepared 26/07/2017

0 87.5 175 350 525 700 Km

Thanks for attention!



STATE VETERINARY INSTITUTE JIHLAVA