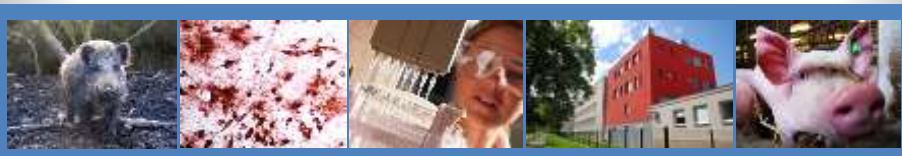
State veterinary institute Jihlava

ASF in the Czech Republic: Sampling and laboratory diagnostics

Petr Vaclavek

NRL for ASF, State Veterinary Institute Jihlava

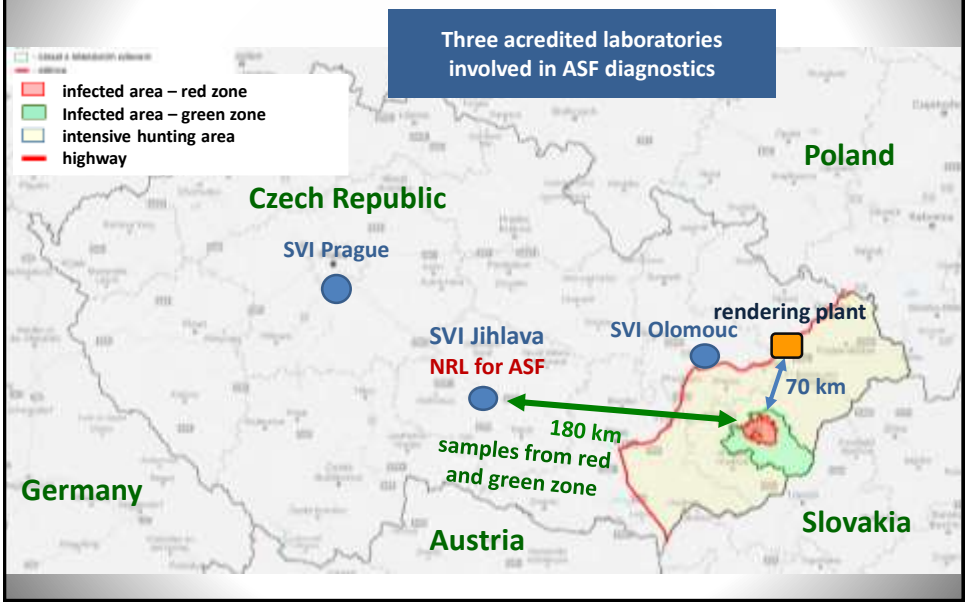
FAO Regional ASF Wild Boar Management Workshop
Belgrade, Serbia, 21-23 May 2019



STATE VETERINARY INSTITUTE JIHLAVA

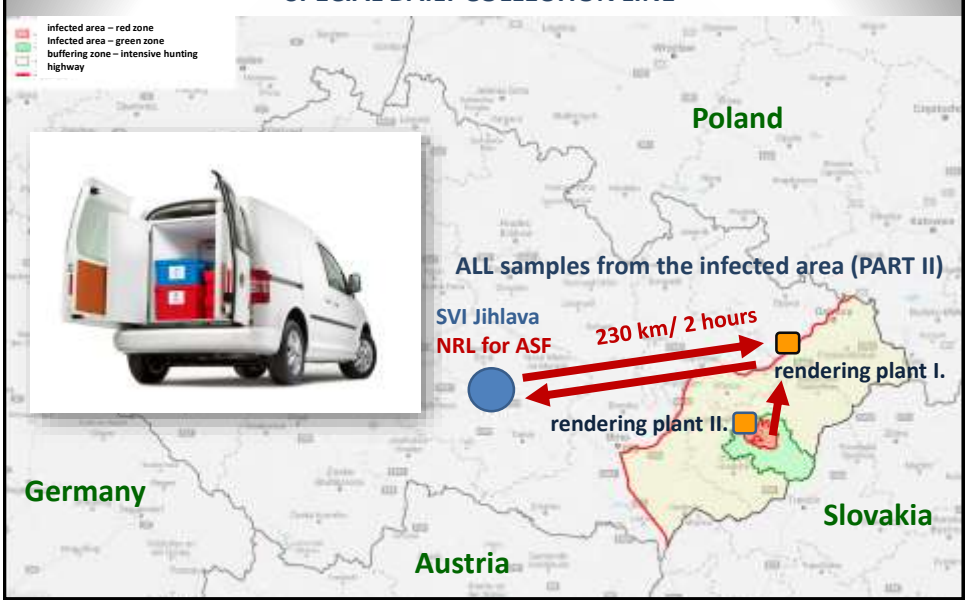
National reference laboratory for CSF and ASF

SVI laboratories in the Czech Republic



Transport of AMP samples from infected area

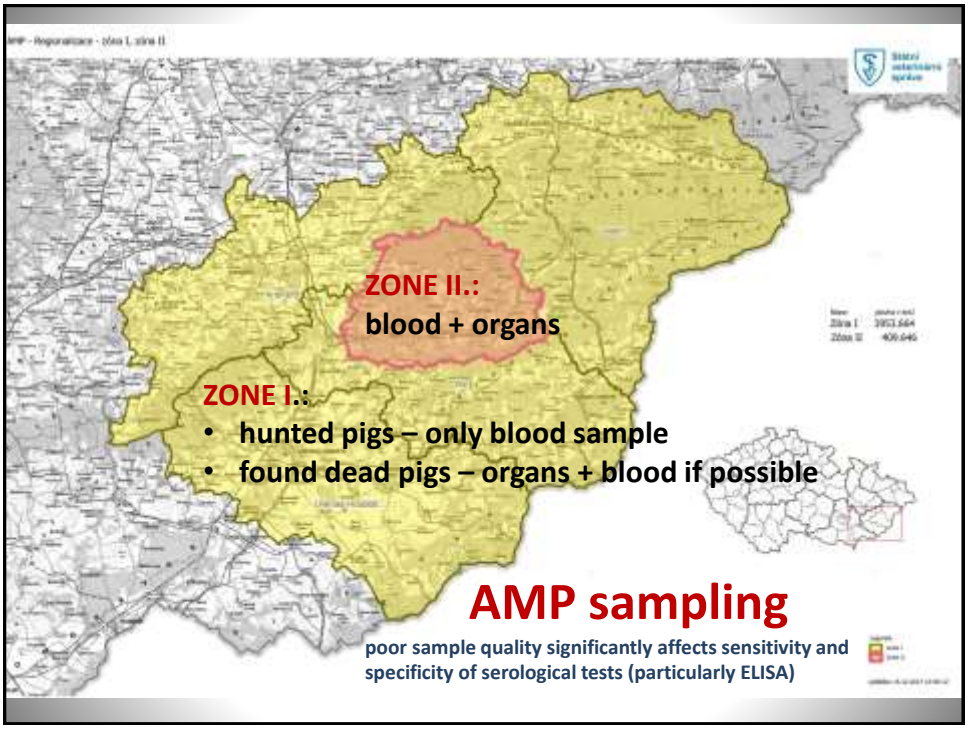
- SPECIAL DAILY COLLECTION LINE -



Sampling: sample types and quality

- **clotted blood** = first choice sample
- **organs** = compulsory in PART II.

(spleen, bone marrow, kidney, lung, tonsils etc.)



Sampling of WB from the INFECTED AREA

NOT in the field!! But ONLY in the rendering plant or in the lab.

- **IMPORTANT BIOSECURITY MEASURE!!!**
- both found carcasses and hunted WB transported into the rendering plant
- samples collection - **authorized veterinarian samples carcasses**
- **DISPOSAL OF CARCASSES IN A RENDERING PLANT**





The largest amount of samples:

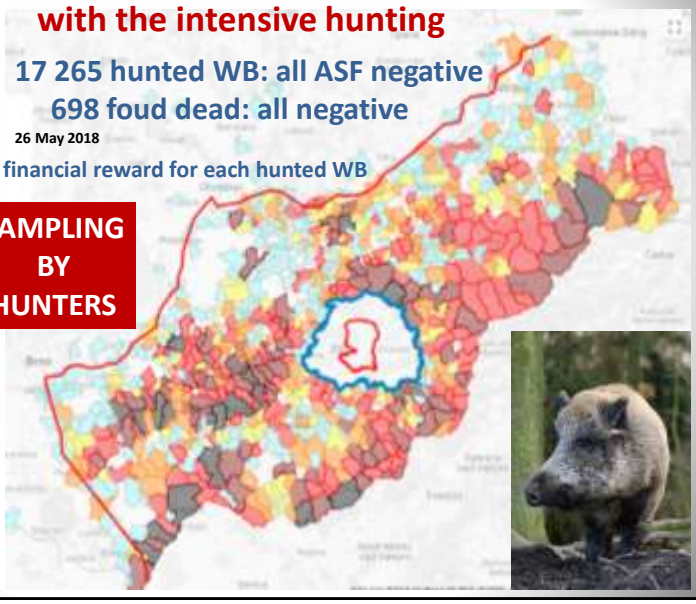
Active and passive surveillance in the in the zone with the intensive hunting

17 265 hunted WB: all ASF negative
698 foud dead: all negative

26 May 2018

financial reward for each hunted WB

SAMPLING BY HUNTERS

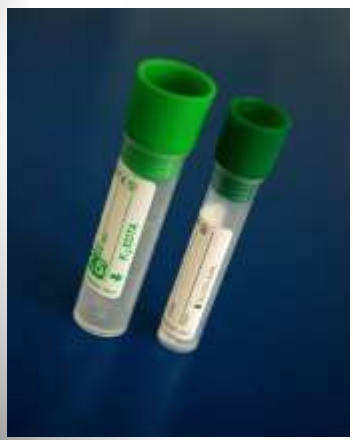


Sample processing in the NRL for ASF




SAMPLING TUBES

BLOOD/ SERUM / PLASMA

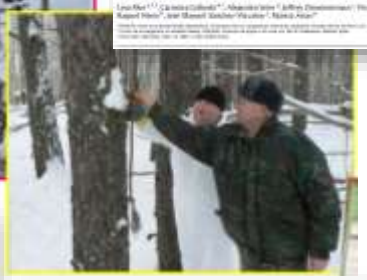

German ASF Sampling Set (Sarstedt)

For the transport by Deutsche Post

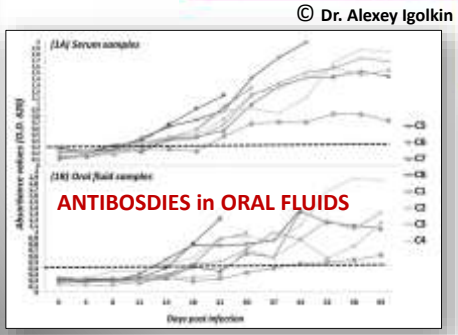
Alternative ways how to collect blood samples for CSF and ASF

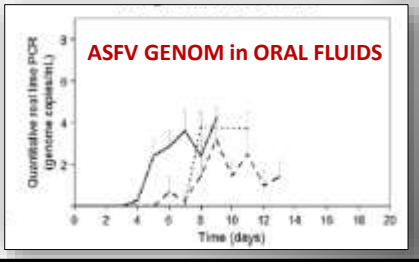
Alternative ASFV sampling methods

ORAL FLUIDS



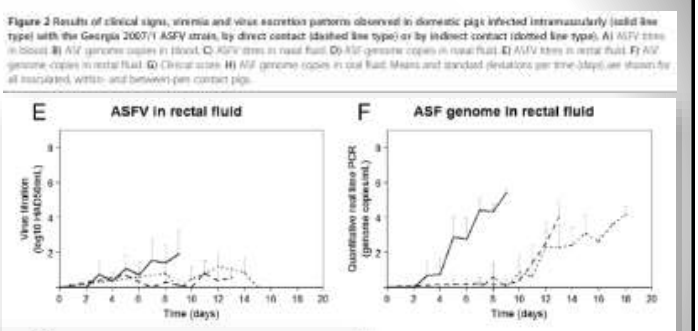
© Dr. Alexey Igolkin



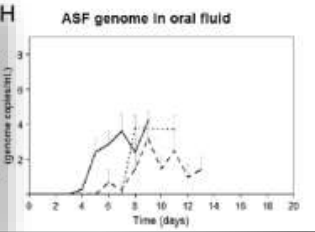


Alternative ASFV sampling methods

FAECAL SAMPLES



Dynamics of African swine fever virus shedding and excretion in domestic pigs infected by intramuscular inoculation and contact transmission



Alternative ASFV sampling methods

BLOOD

SWABS

ORIGINAL ARTICLE

WILEY

Simplifying sampling for African swine fever surveillance: Assessment of antibody and pathogen detection from blood swabs

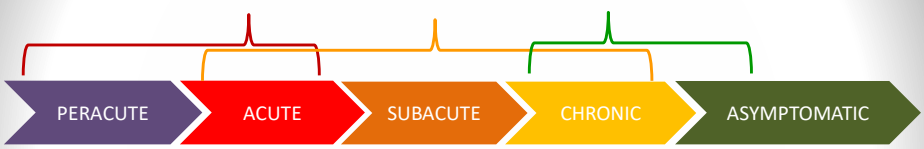
J. Carlson[1] | L. Zani[1] | T. Schwaiger[1] | I. Nurmoja[2,3] | A. Viltrop[3] | A. Vilem[2] | M. Beer[1] | S. Blome[1]

FIGURE 2 GenoTube after dipping in EDTA-treated whole blood (a). Trimming the foam of the swab for testing in multiple assays (b). Pieces of foam cut for DNA extraction (5 mm^2) (c)

CLINICAL FORM vs. LETHALITY vs. VIRULENCE

LETHALITY: 90-100% ↔ 30-50% ↔ 2-10%

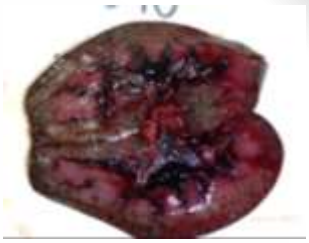
VIRULENCE: HIGH ↔ MODERATE ↔ LOW



CLINICAL FORM is result of many factors:

- ✓ virulence of the strain
- ✓ infectious dose
- ✓ way of infection
- ✓ endemicity
- ✓ breed and condition of pig

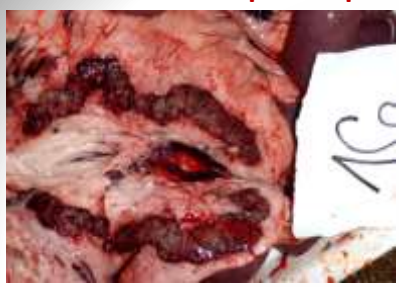
ASF in the Czech Republic: post-mortem lesions in acute form



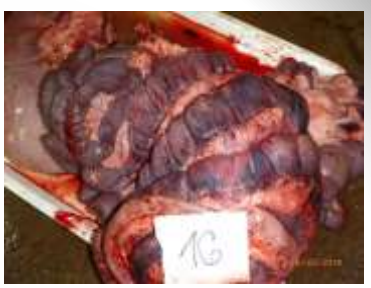
petechiae on kidneys (in cortex and in renal pelvis)

hyperemic splenomegaly (enlarged with rouded edges, friable and dark red to black)

ASF in the Czech Republic: post-mortem lesions in acute form



Lymphnodes: enlarged edematous and completely hemorrhagic similar to a blood clot

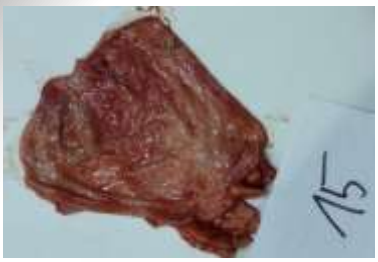


Intestine: petechial haemorrhages on serosa and on mucosa

Lung: petechial haemorrhages

Heart: hydropericardium with redish fluid + petechial haemorrhages on epicardium

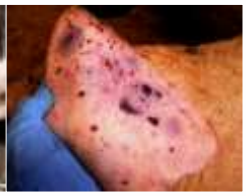
ASF in the Czech Republic: post-mortem lesions in acute form



petechial on urinary bladder

haemorrhages on serosa

© MVDr. František Kostka, MVDr. Kateřina Brázdová



Necrotic areas on the skin surface, subcutaneous haematomas (ears, chest, abdomen and both front and hind legs), reddening of the skin,… etc.

CLINICAL SIGNS AND POST-MORTEM LESIONS ARE INSIGNIFICANT!

Sample logistic and pretreatment

- **preparation** of tissue samples suspension and blood sample purification are necessary BUT **time consuming procedures**
- **acceleration of the process:** e.g. homogenisation of tissue samples (10% wt/vol) – speed-up due the grinding homogenisator Omni Bead Ruptor (24 samples per 2min.)

DNA extraction

MagNA PURE LC a **MagNA PURE 96 (Roche)**

+ compatible commercial extraction kits

MagNA Pure LC Total Nucleid Acid Isolation Kit

MagNa Pure 96 DNA and Viral NA Small Volume Kit (Roche)

PCR methods vs. laboratory capacity

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

= FAST, SENSITIVE, QUANTITATIVE, CLOSED SYSTEMS

E.g. enhancement of lab capacity due the **robotic extraction** of DNA:

- **MagNA PURE (Roche)** – 32/96 samples/1-2 hr
- **QIA Symphony (Qiagen)** – 96 samples/3 hr

+ increase of Real-Time PCR systems: e.g. CFX96 Touch Real-Time PCR (BIO-RAD) = 96 samples/70 min.

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 collective 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

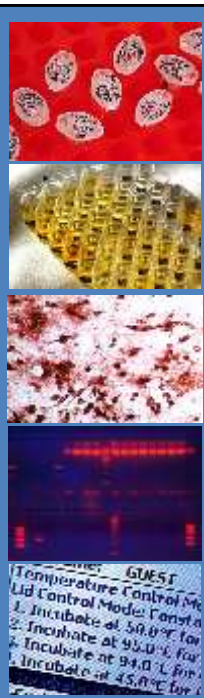
ASF DIAGNOSTICS TESTS used in CZECH LABORATORIES

ANTIBODY DETECTION TECHNIQUES

TEST	TYPE	REFERENCE
ELISA test	INGEZIM PPA Compac blocking ELISA	INGENASA
	ID Screen Indirect ELISA	ID.VET
	ID Screen Competition ELISA	ID.VET
	SVANOVIR® ASFV-Ab indirect ELISA -	Svanova
IPT test	Indirect immunoperoxidase test (IPT)	Gallardo et al. 2013

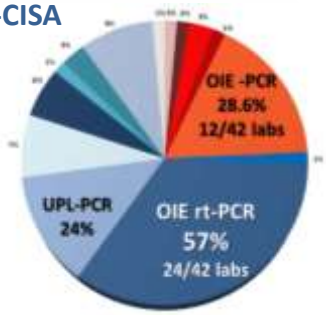
DETECTION of the ASF VIRUS GENOME by PCR

TEST	TYPE	REFERENCE
Conventional PCR	OIE conventional PCR	Agüero et al. 2003
Real Time PCR	UPL Real-time PCR (UPL Probe)	Fernandez et al. 2013
	Taqman Probe (OIE - Real Time PCR)	King et al. 2003 Zsak et al. 2005
	ID Gene ASF Duplex qPCR	ID.vet Innovative Diagnostics



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

PCR tests: Sensitivity and Specificity

Virological diagnosis of African swine fever—Comparative study of available tests

C.A.L. Oura [b,*], L. Edwards [a], C.A. Batten [a]

[a] Institute for Animal Health, Pirbright, Woking, Surrey GU24 0NF, UK
[b] School of Veterinary Medicine, University of the West Indies, St Augustine, Trinidad and Tobago

Table 1
Real-time PCR, Linear-After-The-Exponential-PCR (LATE-PCR) and isothermal assays for the detection of African swine fever virus.

Reference	Detection method	Target	Internal control	Analytical Sensitivity (no. of copies)	Validation (no. of diverse ASFV isolates)	Validation (no. of experimental and field samples)
King et al. (2003)	Real-time PCR (TaqMan)	VP72	Artificial template (mimic)	10–100	41	None
Zsak et al. (2005)	Real-time PCR (TaqMan)	VP72	None	1.4 to 8.4	48	Samples from 6 experimentally infected pigs
McKillen et al. (2010)	MGB probe PCR	9GL gene	None	20	15	Samples from 6 experimentally infected pigs
Tignon et al. (2011)	Real-time PCR (TaqMan)	VP72	β-Actin	5.7–57	44	170 field samples 111 experimental samples
Fernández-Pinero et al. (2012)	UPL probe PCR	VP72	β-Actin	10	40	260 field samples
Rowlands et al. (2011)	LATE-PCR assay	VP72	None	1–10	10	Tissue samples from experimentally infected pigs
Fiorentini et al. (2006)	InvaderR isothermal assay	VP72	No control	2500	1	None
James et al. (2010)	LAMP isothermal assay	Topoisomerase II gene	No control	330	38	Samples from 7 experimentally infected pigs

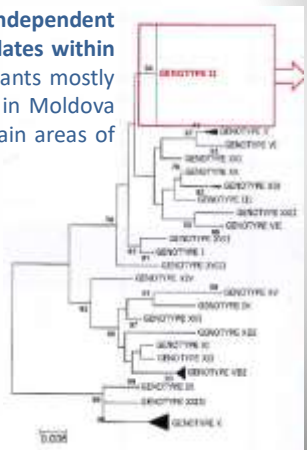
MGB, minor groove binder; UPL, Universal Probe Library; LATE, Linear-After-The-Exponential; LAMP, loop-mediated isothermal amplification.

Molecular characterisation of the Czech ASFV isolates

(EURL for ASF, INIA-CISA)

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

Further subtyping throughout the analysis of three independent ASFV genome regions, clustered the Czech Republic isolates within the CVR-I, IGR-2 and MGF1 variants. These are the variants mostly circulating within the EU countries as well as described in Moldova (2016), Ukraine (2012, 2015), Belarus (2013) and in certain areas of the Russian Federation.

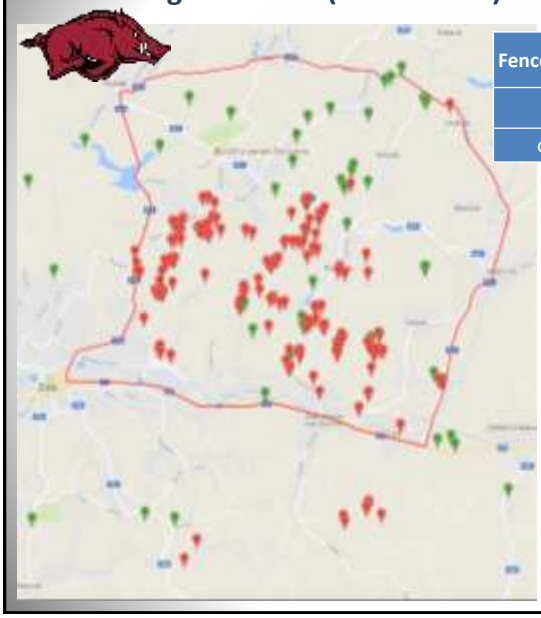


Molecular characterisation of the Czech and others Eastern European ASFV isolates from years 2007-2017

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	2007-2012	II	CVR1	IGR-1	MGF -1
Federation	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 + CVR1SNP* + 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-1 + IGR-2	MGF -1
Moldova	2016	II	CVR1	IGR-2	MGF -1
Czech Republic	**2017**	**II**	**CVR1**	**IGR-2**	**MGF -1**
Romania	2017	II	CVR1	IGR-2	MGF -1

Passive surveillance: wild boars found dead

high risk area (fenced area) inside the infected area



21 May 2018

Fenced area	total	negat.	posit. (virus/PCR)	prevalence
in	280	79	201	71,7%
out	134	123	11	-

WB density in the fenced area:
more than 520 (found dead+hunted)
WB / 57 km2 = 9.1 WB per 1 km2

Nearly 30% negative for ASFV (PCR)

Cause of death ?

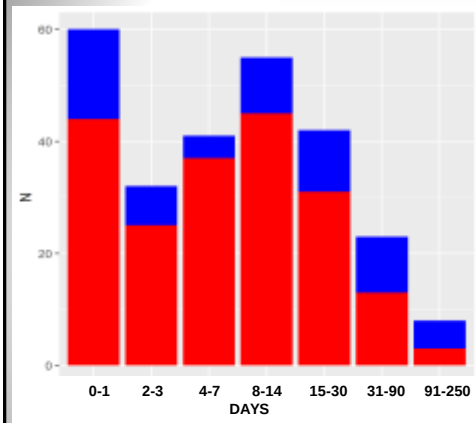
- road-kills
- other diseases
- natural mortality
- „old“ carcasses - skulls and bones

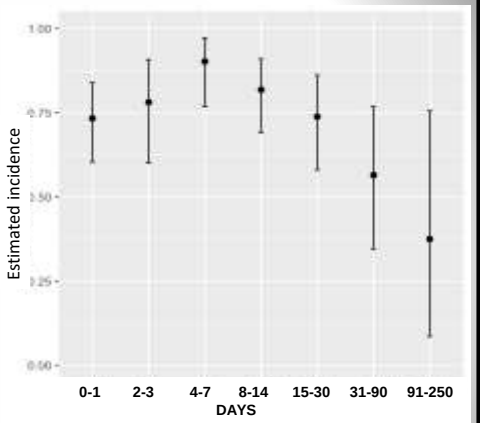
Carcass removal time vs. laboratory result




Carcass removal time

Estimated „age“ of carcasses (days) vs. lab result





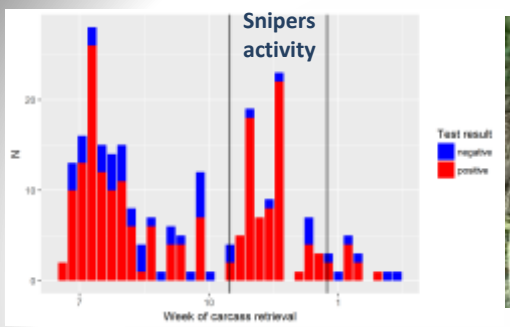
PCR result:

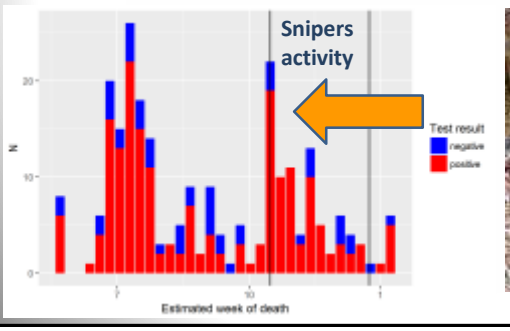
positive

negative

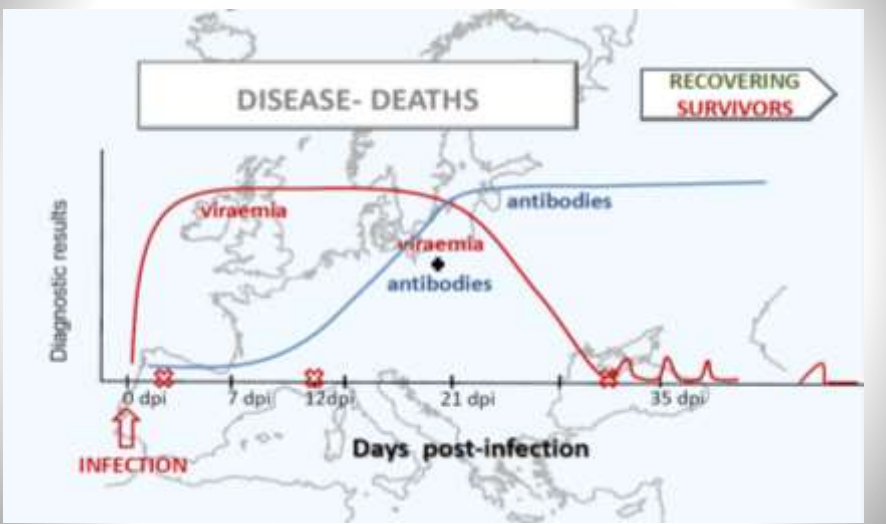


Carcasses „age“ – date of finding vs. date of death (estimated)





ASF infection dynamics: viremia and seroconversion



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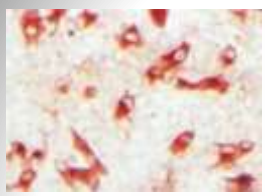
WB positive cases: virology / serology



Positive ASF results: 26 June 2017 – December 2018				
WB	both PCR and ELISA (IPMA) positive	only PCR positive	only ELISA (IPMA comfirmed) positive	Total positive cases
Found dead	10	201	3	214
Hunted	9	9	18	34
TOTAL	19	210	21	250

Wild boars	ASF Virus (PCR)	ASF antibodies (ELISA, IPMA)
Found dead	211	13
Hunted	18	27
TOTAL	229	40

Recovering „survivors“

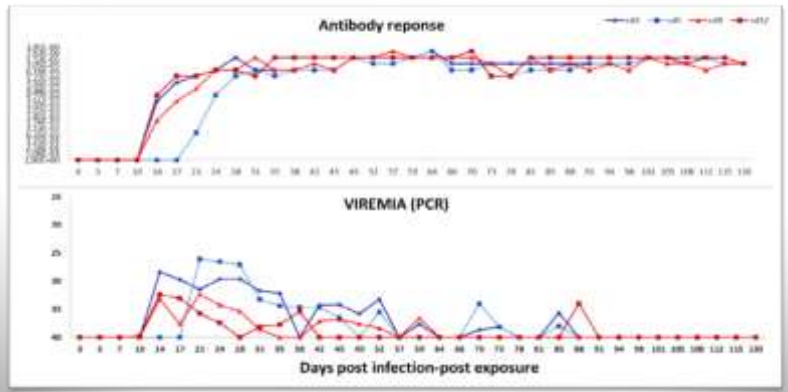
- piglets / adults (1:1)
- 7,2 % ???
- chronic infection?
- virus carriers?

SURVIVORS = potential infection CARRIERS

iinfection with an INTERMITENT VIREMIA may PERZIST for several months without apparent clinical symptoms.

Only laboratory tests can confirm transient / intermittent viremia.

Detection of specific antibodies can reveal these survivors.



ASF serology (detection of Ab) in the CR

In 2017/2018: **606 (74%) found dead WB a 8769 (85%) hunted WB (PART I. and II.) were tested for presence of specific antibodies** (ELISA, IPMA) against ASFV in the Czech Republic (8 April 2018)

- **anti-ASFV antibodies appear soon after infection (7-8 dpi) and persist for up to several months or even years.**
- **serology is crucial for detection of survivors/animals recovered from infection and chronic a subclinical forms!**
- **when a moderate virulent virus isolates are circulating (e.g Estonia) = presence of survivors is significantly increasing**
- the search for antibodies from hunted or dead animals is **essential for obtaining a complete picture of the epidemiology** and also crucial to determine the time of infection

ASF ANTIBODY DETECTION TESTs

Comparative ELISA test study using WB sera with false positive results using the INGENASA ELISA kit

ELISA Results of ELISA tests in the analysis of 243 negative WB SAMPLES

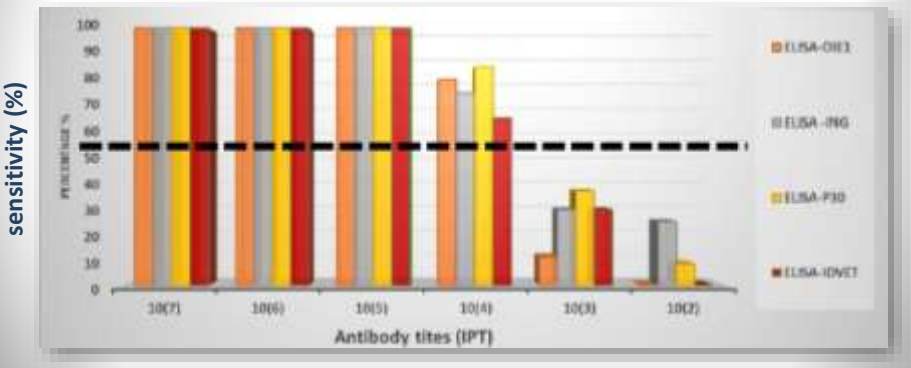
		FALSE POSITIVE RESULTS WB SAMPLES ELISA TESTS							
	Nº SERA	INGENASA ELISA		OIE ELISA		SVANOVA		IDVET	
		NºFP	%FP	NºFP	%FP	NºFP	%FP	NºFP	%FP
POLAND	145	69	47,6	7	4,8	28	19,3	0	0,0
BELGIUM	13	7	53,8	3	23,1	5	38,5	0	0,0
SPAIN	85	13	15,3	4	4,7	7	8,2	0	0,0
TOTAL	243	89	37	14	6	40	16	0	0

SPECIFICITY ELISA TESTs	
IDVET	100%
OIE-ELISA	94%
SVANOVA	84%
INGENASA	63%

The **IDVET ELISA** test provided the **best specificity** in the analysis of negative WB samples

Sensitivity and specificity of serology

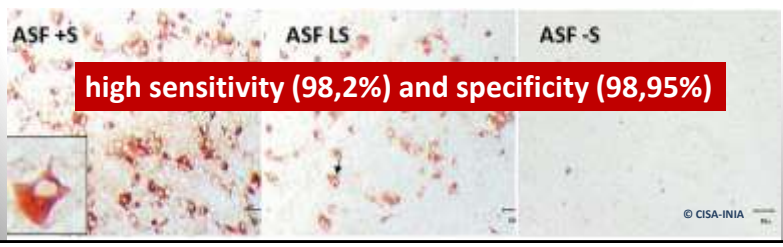
The percentage of sensitivity of ELISA decreases when sera with IPT titres < 10(5) are tested



IPT cut-off is 1:40,
BUT **ELISA reliably detect samples with IPT titres > 1:10 000**

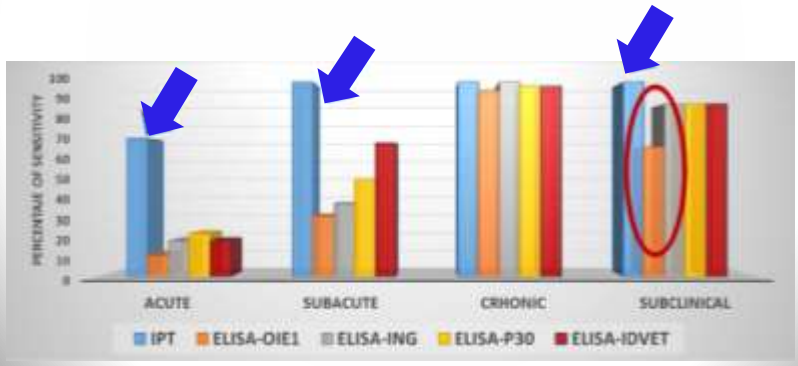
Indirect immunoperoxidase test (IPT)

- **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**
- **CONFIRMATORY TECHNIQUE for positive and doubt ELISA results**
- **samples: sera, exudate tissue, dried blood filter paper sample**



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 screen for ASF Ab, however it should be kept in mind the limitations of the ELISA test



Conclusions

- the Czech Republic has SUFFICIENT CAPACITY for laboratory testing in case of multiple ASF outbreaks
- laboratories are using suitable diagnostic TESTS RECOMMENDED by the EURL
- further studies for evaluation of the relationship of sample quality and final result are needed
- the results found by serological testing confirm the IMPORTANCE OF ANTIBODY DETECTION in the ASF surveillance

Thank you for your attention.