ASF in the Czech Republic: experience from the NRL

Petr Vaclavek, Pavel Bartak

NRL for ASF, State Veterinary Institute Jihlava

Training session on African swine fever under the GF-TADs and European Commission umbrella,
Prague 13 March 2019
Accredited laboratories involved in ASF diagnostics in the Czech Republic

- Infected area – red zone
- Infected area – green zone
- Buffering zone – intensive hunting
- Highway

Map of the Czech Republic showing locations of accredited laboratories involved in ASF diagnostics and nearby countries.
STATE VETERINARY INSTITUTE JIHLAVA

National reference laboratory for CSF and ASF
ZONE II.: blood + organs

ZONE I.:  
- hunted pigs – only blood sample  
- found dead pigs – organs + blood if possible

ASF sampling  
Sample quality could affect sensitivity and specificity of virological and serological tests (particularly PCR and ELISA)
Sampling: sample types and quality

- **clotted blood** = first choice sample
- **ZONE I.** only blood
- **ZONE II.** blood + organs (spleen, bone marrow, kidney, lung, tonsils etc.)
Increased passive surveillance of dead WB – motivated searching for carcases
ASF in the Czech Republic: post-mortem lesions in acute form

petechiae on kidneys (in cortex and in renal pelvis)

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

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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

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

petechial on urinary bladder

haemorrhages on serosa

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CLINICAL SIGNS AND POST-MORTEM LESIONS ARE INSIGNIFICANT!
Sample processing in the NRL for ASF
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: 4x CFX96 Touch Real-Time PCR (BIO-RAD) = 4 x 96 samples/70 min.
Approximate capacity of Czech NRL for ASF (samples tested/1day)

<table>
<thead>
<tr>
<th></th>
<th>Real Time PCR</th>
<th>ELISA Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>blood</td>
<td>tissue</td>
</tr>
<tr>
<td><strong>standard mode</strong></td>
<td>500</td>
<td>300</td>
</tr>
<tr>
<td><strong>crisis mode</strong></td>
<td>1000</td>
<td>500</td>
</tr>
</tbody>
</table>
Approximate collective capacity of all Czech SVI laboratories
(samples tested/1day)

<table>
<thead>
<tr>
<th></th>
<th>Real Time PCR</th>
<th>ELISA Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>standard mode</strong></td>
<td>blood</td>
<td>tissue</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>650</td>
</tr>
<tr>
<td><strong>crisis mode</strong></td>
<td>1900</td>
<td>1000</td>
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</tbody>
</table>
# ASF DIAGNOSTICS TESTS
## used in CZECH LABORATORIES

## ANTIBODY DETECTION TECHNIQUES

<table>
<thead>
<tr>
<th>TEST</th>
<th>TYPE</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA test</td>
<td>INGEZIM PPA Compac blocking ELISA</td>
<td>INGENASA</td>
</tr>
<tr>
<td></td>
<td>ID Screen Indirect ELISA</td>
<td>ID.VET</td>
</tr>
<tr>
<td></td>
<td>ID Screen Competition ELISA</td>
<td>ID.VET</td>
</tr>
<tr>
<td></td>
<td>SVANOVIR® ASFV-Ab indirect ELISA</td>
<td>Svanova</td>
</tr>
<tr>
<td>IPT test</td>
<td>Indirect immunoperoxidase test (IPT)</td>
<td>Gallardo et al. 2013</td>
</tr>
</tbody>
</table>

## DETECTION of the ASF VIRUS GENOME by PCR

<table>
<thead>
<tr>
<th>TEST</th>
<th>TYPE</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional PCR</td>
<td>OIE conventional PCR</td>
<td>Agüero et al. 2003</td>
</tr>
<tr>
<td>Real Time PCR</td>
<td>UPL Real-time PCR (UPL Probe)</td>
<td>Fernandez et al. 2013</td>
</tr>
<tr>
<td></td>
<td>Taqman Probe (OIE - Real Time PCR)</td>
<td>King et al. 2003</td>
</tr>
<tr>
<td></td>
<td>ID Gene ASF Duplex qPCR</td>
<td>ID. VET</td>
</tr>
</tbody>
</table>
**WB positive cases: virology / serology**

### Positive ASF results: 26 June 2017 – October 2018

<table>
<thead>
<tr>
<th>WB</th>
<th>both PCR and ELISA (IPMA) positive</th>
<th>only PCR positive</th>
<th>only ELISA (IPMA confirmed) positive</th>
<th>Total positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found dead</td>
<td>10</td>
<td>201</td>
<td>3</td>
<td>214</td>
</tr>
<tr>
<td>Hunted</td>
<td>9</td>
<td>9</td>
<td>18</td>
<td>34</td>
</tr>
<tr>
<td>TOTAL</td>
<td>19</td>
<td>210</td>
<td>21</td>
<td>250</td>
</tr>
</tbody>
</table>

### Wild boars

<table>
<thead>
<tr>
<th>Wild boars</th>
<th>ASF Virus (PCR)</th>
<th>ASF antibodies (ELISA, IPMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found dead</td>
<td>211</td>
<td>13</td>
</tr>
<tr>
<td>Hunted</td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>TOTAL</td>
<td>229</td>
<td>40</td>
</tr>
</tbody>
</table>

**Recovering „survivors“**

- piglets / adults (1:1)
- 7,2 % ???
- chronic infection?
- virus carriers?
Current situation: summer and autumn 2018

- last 2 serologically positive cases in WB
- hunted WBs in the fenced area

- ASF Ab pozit. (ELISA + IPT)
- ASF DNA negat. (PCR)

18.7.2018
17.10.2018
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
Indirect immunoperoxidase test (IPT)

- confirmatory technique for positive and doubtfull ELISA results
- specific antibody detection on kidney monkey cells infected with adapted virus
- Spanish isolate ASFV Ba71VR adapted on VERO cells
- samples: serum, 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**
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 for ASF Ab screening, however it should be kept in mind the limitations of the ELISA test
Passive surveillance: wild boars found dead high risk area (fenced area) inside the infected area

21 May 2018

<table>
<thead>
<tr>
<th>Fenced area</th>
<th>total</th>
<th>negat.</th>
<th>posit. (virus/PCR)</th>
<th>prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>in</td>
<td>280</td>
<td>79</td>
<td>201</td>
<td>71,7%</td>
</tr>
<tr>
<td>out</td>
<td>134</td>
<td>123</td>
<td>11</td>
<td>-</td>
</tr>
</tbody>
</table>

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?
- car accidents
- other diseases
- natural mortality
- old carcasses skulls and bones
Carcass removal time

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

PCR result:
- **positive**
- **negative**
Conclusions

✓ Czech republic has sufficient capacity for laboratory testing in case of multiple ASF outbreaks
✓ The 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
✓ In total 250 positive cases of ASF in WB were detected (PCR and serology)
✓ The results found by serological testing confirm the importance of antibody detection in the ASF surveillance
Thank you for your attention.