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## African Swine Fever situation in Lithuania

S. Pileviciene, V. Jurgelevicius, J. Buitkuvienė, R. Zagrabskaite, G. Pridotkas

*National Food and Veterinary Risk Assessment Institute*

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On 24th January 2014 Lithuania notified two primary cases of African swine fever (ASF) in wild boar: one male animal, 12 months old, hunted on 22 of January 5 km from the border to Belarus and one female, 3 years old found dead on 20 January 2014 about 40 km north from the border to Belarus. The distance between the two animals was about 36 km. The animals were tested positive for ASF virus (ASFV) genome by real time PCR at the National Reference Laboratory for ASF in Lithuania (NRL). The results were confirmed by the European Reference Laboratory for ASF (CISA-INIA, Madrid, Spain). Intensive wild and domestic animal monitoring programme was started. First positive ASF in a large (~20000 pigs were kept) commercial pig holding with the highest biosecurity confirmed on 23rd of July 2014 in Ignalina district. 290 pigs were sampled from infected farm, and 102 were found positive. 19 217 pigs were killed and destroyed by burying on the territory of the farm. And then ASFV outbreak territory expanded to middle of Lithuania. During this period ~24000 animals were tested for ASF (>10000 domestic pigs, >13000 wild boar). In 2015 were tested 25679 domestic pig (13 were positive) and 24188 wild boar (132 were positive) and in 2016 till 1 of June were tested 1887 domestic pig, 13547 wild boar samples (118 were positive). During 2014-2015 the Institute has received 32 food samples (meat products) confiscated on the border with Belarus, and found 4 of these to be positive for ASFV. The methods used for ASFV detection included highly specific real-time PCR, enzyme-linked immunosorbent assay (ELISA) Ab and Ag, immunoperoxidase test (IPT) (since 2015) and pathological examination.

## P28

## Validation of new Elisa &amp; PCR for the diagnosis of African Swine Fever

Loïc Comtet<sup>1</sup>, Mickael Roche<sup>1</sup>, Gautier HOURDOIR<sup>2</sup>, Séphanle Vêrlité<sup>1</sup>, Fabien Donnet<sup>1</sup>, Matthieu LAFFONT<sup>2</sup>, Lise GREWIS<sup>2</sup>, Philippe Pourquier<sup>1,2</sup>

<sup>1</sup>IDvet, France – <sup>2</sup>IDvet Genetics, France

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African Swine Fever Virus (ASFV) is a highly virulent swine disease characterized by fever, hemorrhages and high mortality rates. ASF control and eradication programs require accurate and reliable diagnostic tests. IDvet offers indirect ELISAs based on three recombinant ASFV antigens (P32, P62, and P72) for antibody detection in serum, blood filter paper and meat juice samples. This ELISA demonstrates excellent specificity, especially for wild boar samples. IDvet has also developed two new tools, a competitive ELISA and a real-time qPCR, for ASF diagnosis. The ID Screen® African Swine Fever Competition ELISA allows for the detection of anti-ASFV P32 antibodies. Specificity was evaluated through the analysis of 280 disease-free sera from domestic and Iberian pigs. Measured specificity was 100.0% (CI 95%: 98.7% - 100.0%). 8 positive reference sera from the ASF European Reference Laboratory (EURL-ASF, Madrid, Spain) were correctly identified as positive. Seroconversion was detected between 6 and 13 dpi. The test correctly identified sera from all genotypes tested, including genotype II. The test was also evaluated by the EURL. Results indicate a diagnostic specificity of 99.4% (n=177) and a diagnostic sensitivity of 95.8% (n=213). Perfect agreement (k=0,95) with the IPT (Immunoperoxidase test) was obtained. The ID Gene™ ASF is a TaqMan ready-to-use real-time PCR assay based on the simultaneous detection of ASFV and an endogenous internal positive control. It may be used for blood, serum, plasma, swabs and tissues samples. Results may be obtained in less than two hours (extraction in only 20 minutes, and amplification around 1 hour). DNA sample panel from the EURL was tested. The ID Gene™ ASF kit correctly identified all samples (14/14, including DNAs from genotypes I, II, V, VIII, IX, X) and did not show any cross-reactions with 31 other pathogens. The detection limit of the PCR was <10 copies, indicating high sensitivity. The EURL-ASF reference panel consisting of 16 ASF lyophilised samples including experimental and clinical field samples, was also tested. DNA extraction was performed by magnetic beads (IDGene™ Mag Fast) as per manufacturer's instructions. All samples were correctly scored positive and negative. To conclude, IDvet offers a full range of tools for the accurate and rapid diagnosis of African Swine Fever, either by serology or qPCR.