Puławy, 29.05.2014 r.

Potwierdzenie III przypadku ASF w Polsce przez EURL

W dniu 29.05. br. Europejskie Laboratorium Referencyjne (EURL) ds. ASF w Valdeolmos w Hiszpanii potwierdziło wynik dodatni uzyskany przez Krajowe Laboratorium Referencyjne ds. ASF w PIWet-PIB w Puławach w próbce pochodzącej od padłego dzika znalezionego w granicznej rzece Świsłocz, w pobliżu miejscowości Rudaki, gmina Krynki, powiat sokólski (certyfikat w załączeniu).

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African Swine Fever (ASF) diagnosis report.

PRELIMINARY REPORT issued by CISA

DATE: 29th May 2014.

ARRIVAL DATE TO CISA: May 28th, 2014.

RESPONSIBLE FOR SUBMISSION:

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Num. ARRIVAL REGISTER CISA: REG. 115/2014

TEST REQUESTED: AFRICAN SWINE FEVER (ASF) CONFIRMATORY DIAGNOSIS.

Num. SAMPLES RECEIVED: samples collected from wild boar identified as;

Sample No	Sample ID
1	DNA NVRI spleen
2	DNA NVRI kidney
3	DNA NVRI blood
4	DNA NVRI lung
5	Homogenised NVRI spleen
6	Homogenised NVRI kidney
7	Homogenised NVRI lung
8	Homogenised CISA spleen
9	Homogenised CISA kidney
10	Homogenised CISA LUNG
11	Blood

EXECUTION DATE: May 28th, 2014.

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ASF DIAGNOSTIC TESTS PERFORMED:

1. ***ASF virological diagnosis:***

   I. ***ASF genome detection.*** A 10% (w/v) clarified homogenized spleen, kidney and lung suspensions were prepared in phosphate-buffered saline [PNT/CISA/PPA/MUESTRAS/1]. The DNA was extracted directly from the undiluted and diluted (1:10) homogenates tissues and from blood using the High Pure PCR Template Preparation Kit [Ref. 11796828001 (ROCHE)] following the manufactures procedures [PNT/CISA/PPA/EXTRACCIÓN ADN/1]. For amplification of the ASFV genomic DNA, the OIE-real time [PNT/CISA/PPA/PCR/2] PCR (OIE 2012) and the UPL real time PCR (Fernandez *et* al., 2013) were achieved in the DNAs extracted at CISA-INIA per each sample and in the DNAs received from the NVRI.

   II. ***ASF virus isolation and haemadsorption (HAD) assay*** has been performed on porcine blood monocytes (PBM) according is described in the OIE Manual (OIE 2012). The PBM has been inoculated at a multiplicity of infection (moi) 1:10 with the with the homogenized spleen, lung and kidney suspensions and with blood sample. After inoculation, a preparation of 1% homologous red blood cells in phosphate-buffered saline has been added to each well and incubated at 95% relative humidity with 5% CO_2, at 37°C. The plates are going to be examined for haemadsorption over a period of seven days.

2. ***ASF serological diagnosis.*** For ASF antibody detection **the spleen, kidney, lung exudates and the blood sample** were tested using the indirect immunoperoxidase technique (IPT) [PNT/CISA/PPA/IPT/1].

3. ***ASFV molecular characterization.*** Genetic characterization of ASF virus from spleen and blood samples has been achieved by PCR and further sequencing analysing three independent regions of ASFV genome internationally validated for the genotyping of ASF isolates. This comprises, i) the C- terminal end of VP72 coding protein gene, which differentiates up to 22 distinct genotypes (Boshoff *et* al., 2007), ii) the full genome sequence of the *p54*-gene (Gallardo *et* al., 2009) and iii) the central variable region (CVR) within the *B602L*-gene (Gallardo *et* al., 2011). **The ASFV genotype II European isolates, from Belarus (Bel13/Grodno) and Poland (Pol14/Krus) have been included as controls.**

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RESULTS

1. ***ASF virological diagnosis:***

   I. ***ASF genome detection*** → **POSITIVE ASF RESULT** has been obtained by **the real time PCRs in ALL SAMPLES TESTED.** The detailed results are summarized in the **table 1**.

Table 1. *ASF virological results obtained on samples received from Poland*

Sample No	Sample ID	OIE-real time PCR (a)		UPL- real time PCR (b)	
		Ct value	Result	Ct value	Result
1	DNA NVRI spleen	19.25	POS	20.96	POS
2	DNA NVRI kidney	20.6	POS	21.54	POS
3	DNA NVRI blood	24.19	POS	26.8	POS
4	DNA NVRI lung	21.26	POS	22.72	POS
5	Homogenised NVRI spleen	19.83	POS	18.85	POS
6	Homogenised NVRI kidney	19.90	POS	19.60	POS
7	Homogenised NVRI lung	22.24	POS	21.91	POS
8	Homogenised CISA spleen	18.01	POS	18.54	POS
9	Homogenised CISA kidney	21.43	POS	21.43	POS
10	Homogenised CISA lung	22.08	POS	21.42	POS
11	Blood	25.03	POS	25.44	POS

(a) ***OIE- real time PCR*** *→ real time PCR (King et al., 2003) technique as is described in the OIE Manual of diagnosis for ASF (Chapter 2.8.1. OIE seventh edition 2012).* **[PNT/CISA/PPA/PCR/2]**

(b) ***UPL-real time PCR*** *→ Real time PCR test described by Fernández et al 2013 based on the Universal Probe Library (UPL)*

(c) ***VI-HAD*** *→ Virus isolation and haemadsorption test on PBM cells as is described in the OIE Manual of diagnosis for ASF (Chapter 2.8.1. OIE seventh edition 2012).*

   II. ***Virus isolation is on going***

2. ***ASF antibody detection*** → The tissue exudates analysed and the blood sample gave **POSITIVE result for antibodies against ASFV using the IPT test.**

3. ***ASFV molecular characterization*** → The expected ~478 bp and ~558 bp of the C-terminal p72-gene and the full length p54-gene, respectively, has been obtained in samples received. The PCR amplicons are being directly sequencing in order to place the ASFV Poland isolate in one of the defined p72 and p54 genotypes. In addition, and for easy genotyping on the basis of different-sized PCR products, the CVR within the *B602L* gene has been amplified. The samples and their DNAs received from the NVRI yielded an amplicon of ≈200 bp, which corresponded in size to the Belarus and Poland genotype II ASFV isolates, which were also included as controls. Further sequence analysis to confirm these results are on-going.

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CONCLUSION

1. **The presence of ASFV has been confirmed by PCR in the samples received from Poland.**
2. **The presence of ASF antibodies has been confirmed by IPT test in the tissue exudates and blood sample.**
3. **On the basis of the different-sized PCR products obtained by the CVR amplification, the preliminary results indicate that the ASFV isolate from Poland clustered within genotype II.**

In Valdeolmos, Madrid (Spain), May 29th , 2014.

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