Puławy, 19.02.2014 r.

Potwierdzenie I przypadku ASF w Polsce przez EURL

W dniu 18.02. 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 dzika znalezionego w miejscowości Grzybowszczyzna (z I przypadku ASF w Polsce) - certyfikat w załączeniu.

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EN SANIDAD ANIMAL

African Swine Fever (ASF) diagnosis and molecular characterization.

Preliminary report issued by CISA

DATE: February 18th, 2014.

ARRIVAL DATE TO CISA: February 18th, 2014.

RESPONSIBLE FOR SUBMISSION:

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Num. ARRIVAL REGISTER CISA: REG. 33/2014

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

Num. SAMPLES RECEIVED: bone marrow sample and the extracted DNA (NVRI) from one European wild boar identified as follows;

Sample No	Sample ID
1	BONE MARROW
2	DNA NVRI sample

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ASF DIAGNOSTIC TESTS PERFORMED:

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

I. ***ASF genome detection.*** The DNA has been extracted directly from the undiluted and diluted (1:10) bone marrow sample received 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 conventional [PNT/CISA/PPA/PCR/1], the OIE-real time [PNT/CISA/PPA/PCR/2] PCRs (OIE 2012) and the UPL real time PCR (Fernandez *et* al., 2013) has been achieved in the DNA extracted at CISA-INIA and in the undiluted and diluted (1:10) DNA 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 bone marrow sample (8 wells/per sample; 10 μl inoculum per well). 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 bill be examined for haemadsorption over a period of seven days.

2. ***ASFV molecular characterization.*** Genetic characterization of ASF virus from the sample received has been initially achieved by PCR 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 genotype II ASFV East European isolates from Ukraine 2012, Belarus 2013 and Lithuania 2014 has been included as controls.

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RESULTS

1. ***ASF virological diagnosis*** → **Positive ASF result** has been obtained **in the bone marrow sample and its DNA received from the wild boar** by the conventional and real time PCRs. The detailed results are summarized in the **Table 1**.

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

Sample identification	OIE-PCR (a)	OIE-real time PCR (b)		UPL-real time PCR (c)*	
	Result	Ct value	Result	Result	Ct value
1. Bone marrow (pure/diluted)	POS/POS	25.82/28.42	POS/POS	25.19/25.18	POS/POS
2. DNA NVRI (pure/diluted)	POS/POS	26.22/29.73	POS/POS	27.26/30.70	POS/POS

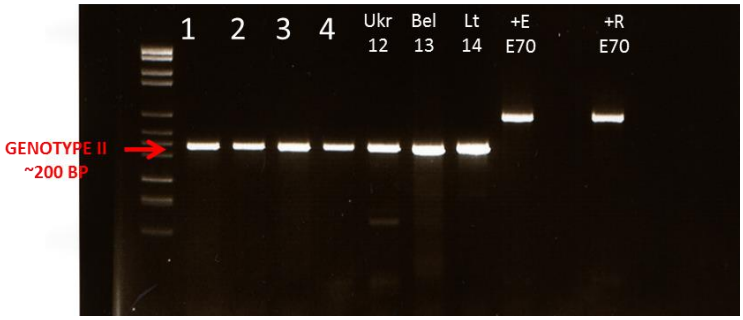
(a) ***OIE-PCR*** → *Conventional PCR (Aguero et al., 2003) technique as is described in the OIE Manual of diagnosis for ASF (Chapter 2.8.1. OIE seventh edition 2012).* **[PNT/CISA/PPA/PCR/1]**

(b) ***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]**

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

2. ***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 the bone marrow sample 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 bone marrow sample and its DNA received from the NVRI yielded an amplicon of ≈200 bp, which corresponded in size to the Ukraine 2012 (Ukr12), Belarus 2013 (Bel13) and Lithuania 2014 (Lt14) genotype II ASFV isolates which were included as controls *(Figure 1).* Further sequence analysis to confirm these results are on-going.

Figure 1; CVR -Subtyping of samples received from Poland identified *as; 1 = bone marrow; 2= bone marrow diluted 1/10; 3 = DNA NVRI; 4 = DNA NVRI diluted 1/10. The positive for the extraction (+E) and for the reaction (+R) correspond to the ASF reference strain E70 belonging to genotype I.*



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CONCLUSION

1. **The presence of ASF has been confirmed in Poland by PCR test in the bone marrow sample received from one European wild boar.**
2. **On the basis of different-sized PCR products obtained by the CVR amplification, the preliminary results of genotyping clustered the virus responsible of the ASF Poland outbreak within genotype II circulating in the Eastern European countries.**

In Valdeolmos, Madrid (Spain), February 18th , 2014.

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