

Puławy 20.10.2016

Potwierdzenie wystąpienia afrykańskiego pomoru świń ;ASF w Polsce przez Europejskie Laboratorium Referencyjne (EURL) ds. ASF w Valdeolmos w Hiszpanii dla przypadku nr 42 oraz przypadków od 75 do 105 oraz ogniska nr 4 (1/2016).



MINISTERIO  
DE ECONOMÍA Y  
COMPETITIVIDAD



Instituto Nacional de Investigación  
y Tecnología Agraria y Alimentaria

SUBDIRECCIÓN GENERAL DE  
INVESTIGACIÓN Y TECNOLOGÍA  
CENTRO DE INVESTIGACIÓN  
EN SANIDAD ANIMAL

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## African Swine Fever (ASF) diagnosis and molecular characterization

### REPORT ISSUED BY THE EUROPEAN UNION REFERENCE LABORATORY FOR AFRICAN SWINE FEVER (EURL-ASF), INIA-CISA

**DATE:** September 23<sup>rd</sup>, 2016.

**ARRIVAL DATE TO CISA:** July 22<sup>nd</sup>, 2016.

**RESPONSIBLE FOR SUBMISSION:**

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**Num. ARRIVAL REGISTER CISA:** REG. 144/2016.

**TEST REQUESTED:** AFRICAN SWINE FEVER (ASF) CONFIRMATORY DIAGNOSIS AND GENE SEQUENCING.

**Num. SAMPLES RECEIVED:** 86 samples from 40 wild boars comprising 20 tissues, 14 homogenates of tissues, 14 bloods and 38 DNAs. 46 samples from 9 domestic pigs comprising 5 bloods, 18 tissues and 23 DNAs identified as follows (table 1):

Table 1 → Identification of the samples received from the NVRI							
HOST	Animals received	Sample ID CISA	Sample laboratory code			TYPE SAMPLE RECEIVED	
			Id code in Poland's NRL		Sampling localization		
WILD BOAR	19121	1	CASE 75	19121/15	Białowieża	TISSUE	BONE MARROW
WILD BOAR	31071/15	2	CASE 79	31071	Narewka	TISSUE	BONE MARROW
WILD BOAR	32842/15	3	CASE 80	32842	Narewka	TISSUE	BONE MARROW
WILD BOAR	32981/15	4	CASE 81	32981	Białowieża	TISSUE	BONE MARROW
WILD BOAR	00969/16	5	CASE 85	00969	Narewka	TISSUE	SPLEEN
WILD BOAR	00969/16	6	CASE 85	00969	Narewka	TISSUE	LYMPHNODE
WILD BOAR	00969/16	7	CASE 85	00969	Narewka	TISSUE	KIDNEY



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WILD BOAR	05778/16	8	CASE 89	05778	Narewka	TISSUE	BONE MARROW
WILD BOAR	06985/16	9	CASE 90	06985	Białowieża	TISSUE	BONE MARROW
WILD BOAR	07631/16	10	CASE 91	07631	Białowieża	TISSUE	BONE MARROW
WILD BOAR	10375/16	11	CASE 93	10375	Narewka	TISSUE	BONE MARROW
WILD BOAR	12990/16	12	CASE 94	12990	Narewka	TISSUE	BONE MARROW
WILD BOAR	14055/16	13	CASE 95	14055	Białowieża	HOMOGENATE	BONE MARROW
WILD BOAR	14056/16	14	CASE 95	14056	Białowieża	HOMOGENATE	BONE MARROW
DOMESTIC PIG	15337/16	15	OUTBREAK 4	15337	Hajnówka	TISSUE	KIDNEY
DOMESTIC PIG	15337/16	16	OUTBREAK 4	15337	Hajnówka	TISSUE	SPLEEN
DOMESTIC PIG	15338/16	17	OUTBREAK 4	15338	Hajnówka	TISSUE	KIDNEY
DOMESTIC PIG	15338/16	18	OUTBREAK 4	15338	Hajnówka	TISSUE	SPLEEN
DOMESTIC PIG	15339/16	19	OUTBREAK 4	15339	Hajnówka	TISSUE	KIDNEY
DOMESTIC PIG	15339/16	20	OUTBREAK 4	15339	Hajnówka	TISSUE	SPLEEN
DOMESTIC PIG	15340/16	21	OUTBREAK 4	15340	Hajnówka	TISSUE	KIDNEY
DOMESTIC PIG	15340/16	22	OUTBREAK 4	15340	Hajnówka	TISSUE	SPLEEN
DOMESTIC PIG	15341/16	23	OUTBREAK 4	15341	Hajnówka	TISSUE	KIDNEY
DOMESTIC PIG	15341/16	24	OUTBREAK 4	15341	Hajnówka	TISSUE	SPLEEN
DOMESTIC PIG	15553/16	25	OUTBREAK 4	15553	Hajnówka	TISSUE	KIDNEY
DOMESTIC PIG	15553/16	26	OUTBREAK 4	15553	Hajnówka	TISSUE	SPLEEN
DOMESTIC PIG	15554/16	27	OUTBREAK 4	15554	Hajnówka	TISSUE	KIDNEY
DOMESTIC PIG	15554/16	28	OUTBREAK 4	15554	Hajnówka	TISSUE	SPLEEN
DOMESTIC PIG	15555/16	29	OUTBREAK 4	15555	Hajnówka	TISSUE	KIDNEY
DOMESTIC PIG	15555/16	30	OUTBREAK 4	15555	Hajnówka	TISSUE	SPLEEN
DOMESTIC PIG	15556/16	31	OUTBREAK 4	15556	Hajnówka	TISSUE	KIDNEY
DOMESTIC PIG	15556/16	32	OUTBREAK 4	15556	Hajnówka	TISSUE	SPLEEN
WILD BOAR	15777/16	33	CASE 96	15777	Czyże	HOMOGENATE	BONE MARROW
WILD BOAR	15778/16	34	CASE 96	15778	Czyże	HOMOGENATE	BONE MARROW
WILD BOAR	15779/16	35	CASE 96	15779	Czyże	HOMOGENATE	BONE MARROW
WILD BOAR	15780/16	36	CASE 96	15780	Czyże	HOMOGENATE	BONE MARROW
WILD BOAR	15780/16	37	CASE 96	15780	Czyże	TISSUE	SPLEEN
WILD BOAR	16080/16	38	CASE 98	16080	Białowieża	HOMOGENATE	BONE MARROW
WILD BOAR	16554/16	39	CASE 99	16554	Hajnówka	HOMOGENATE	BONE MARROW
WILD BOAR	16555/16	40	CASE 99	16555	Hajnówka	TISSUE	SPLEEN
WILD BOAR	16555/16	41	CASE 99	16555	Hajnówka	TISSUE	KIDNEY
WILD BOAR	16557/16	42	CASE 99	16557	Hajnówka	TISSUE	SPLEEN
WILD BOAR	16557/16	43	CASE 99	16557	Hajnówka	TISSUE	KIDNEY
WILD BOAR	16677/16	44	CASE 101	16677		TISSUE	KIDNEY
WILD BOAR	16677/16	45	CASE 101	16677		TISSUE	SPLEEN
WILD BOAR	16858/16	46	CASE 102	16858	Hajnówka	TISSUE	BONE MARROW
WILD BOAR	17013/16	47	CASE 103	17013	Hajnówka	HOMOGENATE	BONE MARROW
WILD BOAR	17014/16	48	CASE 104	17014	Hajnówka	HOMOGENATE	BONE MARROW
WILD BOAR	17015/16	49	CASE 104	17015	Hajnówka	HOMOGENATE	BONE MARROW
WILD BOAR	17016/16	50	CASE 104	17016	Hajnówka	HOMOGENATE	BONE MARROW
WILD BOAR	17107/16	51	CASE 105	17107	Hajnówka	HOMOGENATE	BONE MARROW
WILD BOAR	05097/15	52	CASE 42	05097	Gródek	HOMOGENATE	BONE MARROW
WILD BOAR	19765-17/15	53	CASE 76	19765 - WB Nº 17	Krynki	BLOOD	BLOOD-EDTA
WILD BOAR	27152-55/15	54	CASE 77	27152 - WB Nº 55	Gródek	BLOOD	BLOOD-EDTA
WILD BOAR	30296-10/15	55	CASE 78	30296 - WB Nº 10	Sidra	BLOOD	BLOOD-EDTA
WILD BOAR	33256-22/15	56	CASE 83	33256 - WB Nº 22	Gródek	BLOOD	BLOOD-EDTA
WILD BOAR	33245-3/15	57	CASE 82	33245 - WB Nº 3	Narew	BLOOD	BLOOD-EDTA
WILD BOAR	00347-22/16	58	CASE 84	00347 WB Nº 22	Narew	BLOOD	BLOOD-EDTA
WILD BOAR	00969/16	59	CASE 85	00969	Narewka	BLOOD	BLOOD-EDTA
WILD BOAR	03518-8/16	60	CASE 86	03518 - WB Nº 8	Narewka	BLOOD	BLOOD-EDTA
WILD BOAR	0451/16	61	CASE 87	04513	Supraśl	BLOOD	BLOOD-EDTA
WILD BOAR	05432-12/16	62	CASE 88	05432 WB Nº 12	Białowieża	BLOOD	BLOOD-EDTA
WILD BOAR	08590-1/16	63	CASE 92	08590 - WB Nº r 1	Michałowo	BLOOD	BLOOD-EDTA



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DOMESTIC PIG	15337/16	64	OUTBREAK 4	15337	Hajnówka	BLOOD	BLOOD-EDTA
DOMESTIC PIG	15338/16	65	OUTBREAK 4	15338	Hajnówka	BLOOD	BLOOD-EDTA
DOMESTIC PIG	15339/16	66	OUTBREAK 4	15339	Hajnówka	BLOOD	BLOOD-EDTA
DOMESTIC PIG	15340/16	67	OUTBREAK 4	15340	Hajnówka	BLOOD	BLOOD-EDTA
DOMESTIC PIG	15341/16	68	OUTBREAK 4	15341	Hajnówka	BLOOD	BLOOD-EDTA
WILD BOAR	15887-3/16	69	CASE 97	15887 WB Nº 3	Narewka	BLOOD	BLOOD-EDTA
WILD BOAR	16565-7/16	70	CASE 100	16565 WB Nº 7	Hajnówka	BLOOD	BLOOD-EDTA
WILD BOAR	16677/16	71	CASE 101	16677	Narewka	BLOOD	BLOOD-EDTA
WILD BOAR	19121	72	CASE 75	19121/15	Białowieża	DNA	BONE MARROW
WILD BOAR	31071/15	73	CASE 79	31071	Narewka	DNA	BONE MARROW
WILD BOAR	32842/15	74	CASE 80	32842	Narewka	DNA	BONE MARROW
WILD BOAR	32981/15	75	CASE 81	32981	Białowieża	DNA	BONE MARROW
WILD BOAR	33245-3/15	76	CASE 82	33245- WB Nº 3	Narew	DNA	BLOOD-EDTA
WILD BOAR	00969/16	77	CASE 85	00969	Narewka	DNA	SPLEEN
WILD BOAR	00969/16	78	CASE 85	00969	Narewka	DNA	LYMPHNODE
WILD BOAR	00969/16	79	CASE 85	00969	Narewka	DNA	KIDNEY
WILD BOAR	00969/16	80	CASE 85	00969	Narewka	DNA	BLOOD-EDTA
WILD BOAR	0451/16	81	CASE 87	04513	Supraśl	DNA	BLOOD-EDTA
WILD BOAR	05778/16	82	CASE 89	05778	Narewka	DNA	BONE MARROW
WILD BOAR	06985/16	83	CASE 90	06985	Białowieża	DNA	BONE MARROW
WILD BOAR	07631/16	84	CASE 91	07631	Białowieża	DNA	BONE MARROW
WILD BOAR	10375/16	85	CASE 93	10375	Narewka	DNA	BONE MARROW
WILD BOAR	12990/16	86	CASE 94	12990	Narewka	DNA	BONE MARROW
WILD BOAR	14055/16	87	CASE 95	14055	Białowieża	DNA	BONE MARROW
WILD BOAR	14056/16	88	CASE 95	14056	Białowieża	DNA	BONE MARROW
DOMESTIC PIG	15337/16	89	OUTBREAK 4	15337	Hajnówka	DNA	BLOOD-EDTA
DOMESTIC PIG	15337/16	90	OUTBREAK 4	15337	Hajnówka	DNA	KIDNEY
DOMESTIC PIG	15337/16	91	OUTBREAK 4	15337	Hajnówka	DNA	SPLEEN
DOMESTIC PIG	15338/16	92	OUTBREAK 4	15338	Hajnówka	DNA	BLOOD-EDTA
DOMESTIC PIG	15338/16	93	OUTBREAK 4	15338	Hajnówka	DNA	KIDNEY
DOMESTIC PIG	15338/16	94	OUTBREAK 4	15338	Hajnówka	DNA	SPLEEN
DOMESTIC PIG	15339/16	95	OUTBREAK 4	15339	Hajnówka	DNA	BLOOD-EDTA
DOMESTIC PIG	15339/16	96	OUTBREAK 4	15339	Hajnówka	DNA	KIDNEY
DOMESTIC PIG	15339/16	97	OUTBREAK 4	15339	Hajnówka	DNA	SPLEEN
DOMESTIC PIG	15340/16	98	OUTBREAK 4	15340	Hajnówka	DNA	BLOOD-EDTA
DOMESTIC PIG	15340/16	99	OUTBREAK 4	15340	Hajnówka	DNA	KIDNEY
DOMESTIC PIG	15340/16	100	OUTBREAK 4	15340	Hajnówka	DNA	SPLEEN
DOMESTIC PIG	15341/16	101	OUTBREAK 4	15341	Hajnówka	DNA	BLOOD-EDTA
DOMESTIC PIG	15341/16	102	OUTBREAK 4	15341	Hajnówka	DNA	KIDNEY
DOMESTIC PIG	15341/16	103	OUTBREAK 4	15341	Hajnówka	DNA	SPLEEN
DOMESTIC PIG	15553/16	104	OUTBREAK 4	15553	Hajnówka	DNA	KIDNEY
DOMESTIC PIG	15553/16	105	OUTBREAK 4	15553	Hajnówka	DNA	SPLEEN
DOMESTIC PIG	15554/16	106	OUTBREAK 4	15554	Hajnówka	DNA	KIDNEY
DOMESTIC PIG	15554/16	107	OUTBREAK 4	15554	Hajnówka	DNA	SPLEEN
DOMESTIC PIG	15555/16	108	OUTBREAK 4	15555	Hajnówka	DNA	KIDNEY
DOMESTIC PIG	15555/16	109	OUTBREAK 4	15555	Hajnówka	DNA	SPLEEN
DOMESTIC PIG	15556/16	110	OUTBREAK 4	15556	Hajnówka	DNA	KIDNEY
DOMESTIC PIG	15556/16	111	OUTBREAK 4	15556	Hajnówka	DNA	SPLEEN
WILD BOAR	15777/16	112	CASE 96	15777	Czyże	DNA	BONE MARROW
WILD BOAR	15778/16	113	CASE 96	15778	Czyże	DNA	BONE MARROW
WILD BOAR	15779/16	114	CASE 96	15779	Czyże	DNA	BONE MARROW
WILD BOAR	15780/16	115	CASE 96	15780	Czyże	DNA	BONE MARROW
WILD BOAR	15780/16	116	CASE 96	15780	Czyże	DNA	SPLEEN
WILD BOAR	16080/16	117	CASE 98	16080	Białowieża	DNA	BONE MARROW
WILD BOAR	16554/16	118	CASE 99	16554	Hajnówka	DNA	BONE MARROW
WILD BOAR	16555/16	119	CASE 99	16555	Hajnówka	DNA	SPLEEN



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WILD BOAR	16555/16	120	CASE 99	16555	Hajnówka	DNA	KIDNEY
WILD BOAR	16557/16	121	CASE 99	16557	Hajnówka	DNA	KIDNEY
WILD BOAR	16557/16	122	CASE 99	16557	Hajnówka	DNA	SPLEEN
WILD BOAR	16565-7/16	123	CASE 100	16565 - WB Nº 7	Hajnówka	DNA	BLOOD-EDTA
WILD BOAR	16677/16	124	CASE 101	16677		DNA	BLOOD-EDTA
WILD BOAR	16677/16	125	CASE 101	16677		DNA	KIDNEY
WILD BOAR	16677/16	126	CASE 101	16677		DNA	SPLEEN
WILD BOAR	16858/16	127	CASE 102	16858	Hajnówka	DNA	BONE MARROW
WILD BOAR	17013/16	128	CASE 103	17013	Hajnówka	DNA	BONE MARROW
WILD BOAR	17014/16	129	CASE 104	17014	Hajnówka	DNA	BONE MARROW
WILD BOAR	17015/16	130	CASE 104	17015	Hajnówka	DNA	BONE MARROW
WILD BOAR	17016/16	131	CASE 104	17016	Hajnówka	DNA	BONE MARROW
WILD BOAR	17107/16	132	CASE 105	17107	Hajnówka	DNA	BONE MARROW

**EXECUTION DATE:** From July, 26<sup>th</sup> to September 12<sup>th</sup>, 2016

#### ASF DIAGNOSTIC TESTS PERFORMED:

##### 1. ASF virus detection:

- a. **ASFV genome detection.** A 10% (w/v) clarified homogenized tissue suspensions have been prepared in phosphate-buffered saline [PNT/CISA/PPA/MUESTRAS/1]. The DNA has been extracted directly from the 34 tissue homogenates and 14 bloods from the 40 wild boar and from 18 tissue homogenates and 5 bloods from the 9 domestic pigs received using the High Pure PCR Template Preparation Kit [Ref. 11796828001 (ROCHE)] following the standardised procedure [PNT/CISA/PPA/EXTRACCIÓN ADN/1]. For amplification of the ASFV DNA, the UPL real-time PCR (Fernández-Pinero *et al.*, 2013) [PNT/CISA/PPA/PCR/3] have been performed in the DNAs extracted from blood, tissue homogenates and from the 61 extracted DNAs directly received from the NRL.
- b. **ASF virus isolation and haemadsorption (HAD) assay\*** [PNT/CISA/PPA/VI/1] was performed on porcine blood monocytes (PBM) according is described in the OIE Manual (OIE 2012). The PBM were inoculated at a multiplicity of infection (moi) 1:10 with selected PCR positive samples (8 wells/per sample; 10 µl inoculum per well) in which was obtained a positive PCR result. After inoculation, a preparation of 1% homologous red blood cells in phosphate-buffered saline was added to each well and incubated at 95% relative humidity with 5% CO<sub>2</sub>, at 37°C. The plates were examined for haemadsorption over a period of seven days. The samples have been blind passaged three times.

2. **ASF serological diagnosis.** For ASF antibody detection, **66 samples** included 23 tissue exudates, 19 blood and 24 bone marrow homogenates were tested using the indirect immunoperoxidase technique (IPT)\* [PNT/CISA/PPA/IPT/1] in two fold dilutions starting from 1/20 (in case of bone marrow starting from 1/5).



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3. **ASFV molecular characterization\***. Genetic characterization of ASFV from **23 wild boar and 2 domestic pig** has been performed by PCR and further sequencing throughout the analysis of three independent regions of the ASFV genome which comprises; i) the C-terminal end of VP72 coding protein gene, which differentiates up to 23 distinct genotypes (Boshoff et al., 2007; Achenbach JE et al., 2016), ii) the intergenic region located between the *I73R* and *I329L* genes and characterized by the presence of TRS (Gallardo et al., 2014) and iii) the central variable region (CVR) within the B602L-gene (Gallardo et al., 2011). We have compared the nucleotide sequences obtained with those of previously described representative genotype II ASFVs isolated since April 2007 up to September 2016 from wild and domestic pigs in Eastern and Central European countries (Lithuania, Poland, Latvia, Estonia, Belarus, Ukraine, Armenia, Azerbaijan, Georgia and Russia Federation).

## RESULTS

### 1. ASF VIRUS DETECTION:

- 1.1. **WILD BOAR SAMPLES** → **Positive ASFV result** has been obtained by the UPL real-time PCR in **79 out of 86 samples tested from wild boar confirming the presence of ASFV in 34 out of the 40 wild boar received (85%)**. The weak positive result (Ct>35) obtained in the samples identified as 58 and 60 (ID CISA) was confirmed in a second PCR amplification run in which the extracted DNA per each sample was analysed by duplicate. **After three passages in PAM, the ASFV has been isolated only from 2 wild boar identified as 32981** (ID CISA 4, case 81) **and 07631** (ID CISA 10, case 91) showing the classical haemadsorbing pattern of the ASFV circulating in Europe. The results obtained in virus detection are summarized in the **table 2**.

Sample ID CISA	Sample laboratory code in Poland's NRL		TYPE SAMPLE RECEIVED		UPL-real time PCR <sup>(a)</sup>				VI-HAD <sup>(b)</sup> + (3 passages)
					CT value	2 <sup>nd</sup> PCR CT values	3 <sup>rd</sup> PCR CT values	Result	
1	CASE 75	19121/15	TISSUE	BONE MARROW	28.35			POSITIVE	NEG
2	CASE 79	31071	TISSUE	BONE MARROW	32.75			POSITIVE	NT
3	CASE 80	32842	TISSUE	BONE MARROW	25.56			POSITIVE	NEG
4	CASE 81	32981	TISSUE	BONE MARROW	25.62			POSITIVE	POS
5	CASE 85	00969	TISSUE	SPLEEN	21.34			POSITIVE	NEG
6	CASE 85	00969	TISSUE	LYMPHNODE	32.4			POSITIVE	NT
7	CASE 85	00969	TISSUE	KIDNEY	24.92			POSITIVE	NT
8	CASE 89	05778	TISSUE	BONE MARROW	25.98			POSITIVE	NEG
9	CASE 90	06985	TISSUE	BONE MARROW	27.93			POSITIVE	NEG
10	CASE 91	07631	TISSUE	BONE MARROW	26.01			POSITIVE	POS
11	CASE 93	10375	TISSUE	BONE MARROW	23.01			POSITIVE	NEG



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12	CASE 94	12990	TISSUE	BONE MARROW	25.13			POSITIVE	NEG
13	CASE 95	14055	HOMOGENATE	BONE MARROW	14.79			POSITIVE	NT
14	CASE 95	14056	HOMOGENATE	BONE MARROW	18.69			POSITIVE	NT
33	CASE 96	15777	HOMOGENATE	BONE MARROW	29.38 <sup>(1)</sup>			POSITIVE	NT
34	CASE 96	15778	HOMOGENATE	BONE MARROW	30.5			POSITIVE	NT
35	CASE 96	15779	HOMOGENATE	BONE MARROW	31.34			POSITIVE	NT
36	CASE 96	15780	HOMOGENATE	BONE MARROW	19.36			POSITIVE	NT
37	CASE 96	15780	TISSUE	SPLEEN	24.85			POSITIVE	NT
38	CASE 98	16080	HOMOGENATE	BONE MARROW	21.96			POSITIVE	NT
39	CASE 99	16554	HOMOGENATE	BONE MARROW	33.87			POSITIVE	NT
40	CASE 99	16555	TISSUE	SPLEEN	25.81			POSITIVE	NEG
41	CASE 99	16555	TISSUE	KIDNEY	30.12			POSITIVE	NT
42	CASE 99	16557	TISSUE	SPLEEN	28.94			POSITIVE	NT
43	CASE 99	16557	TISSUE	KIDNEY	No ct			NEGATIVE	NT
44	CASE 101	16677	TISSUE	KIDNEY	26.5			POSITIVE	NT
45	CASE 101	16677	TISSUE	SPLEEN	26.24			POSITIVE	NEG
46	CASE 102	16858	TISSUE	BONE MARROW	24.6			POSITIVE	NEG
47	CASE 103	17013	HOMOGENATE	BONE MARROW	23.85			POSITIVE	NEG
48	CASE 104	17014	HOMOGENATE	BONE MARROW	23.2			POSITIVE	NEG
49	CASE 104	17015	HOMOGENATE	BONE MARROW	26.11			POSITIVE	NT
50	CASE 104	17016	HOMOGENATE	BONE MARROW	30.4			POSITIVE	NT
51	CASE 105	17107	HOMOGENATE	BONE MARROW	30.57			POSITIVE	NT
52	CASE 42	05097	HOMOGENATE	BONE MARROW	28.34			POSITIVE	NEG
53	CASE 76	19765 - WB Nº17	BLOOD	BLOOD-EDTA	No ct <sup>(1)</sup>			NEGATIVE	NT
54	CASE 77	27152 - WB Nº 55	BLOOD	BLOOD-EDTA	No ct			NEGATIVE	NT
55	CASE 78	30296 - WB Nº 10	BLOOD	BLOOD-EDTA	No ct			NEGATIVE	NT
56	CASE 83	33256 - WB Nº 22	BLOOD	BLOOD-EDTA	No ct			NEGATIVE	NT
57	CASE 82	33245 - WB Nº 3	BLOOD	BLOOD-EDTA	26.71			POSITIVE	NT
58	CASE 84	00347 WB Nº 22	BLOOD	BLOOD-EDTA	36.53 <sup>(1)</sup>	36.73	36.64	WEAK	NT
59	CASE 85	00969	BLOOD	BLOOD-EDTA	20.4 <sup>(1)</sup>			POSITIVE	NT
60	CASE 86	03518 - WB Nº 8	BLOOD	BLOOD-EDTA	37.95	38.86	39.64	WEAK	NT
61	CASE 87	04513	BLOOD	BLOOD-EDTA	19.55			POSITIVE	NT
62	CASE 88	05432 WB Nº 12	BLOOD	BLOOD-EDTA	No ct			NEGATIVE	NT
63	CASE 92	08590 - WB Nº 1	BLOOD	BLOOD-EDTA	No ct			NEGATIVE	NT
69	CASE 97	15887 WB Nº 3	BLOOD	BLOOD-EDTA	26.57			POSITIVE	NT
70	CASE 100	16565 WB Nº 7	BLOOD	BLOOD-EDTA	28.25			POSITIVE	NT
71	CASE 101	16677	BLOOD	BLOOD-EDTA	23.88			POSITIVE	NT
72	CASE 75	19121/15	DNA	BONE MARROW	26			POSITIVE	NT
73	CASE 79	31071	DNA	BONE MARROW	29.17			POSITIVE	NT
74	CASE 80	32842	DNA	BONE MARROW	21.14			POSITIVE	NT
75	CASE 81	32981	DNA	BONE MARROW	25.16			POSITIVE	NT
76	CASE 82	33245 - WB Nº 3	DNA	BLOOD-EDTA	26.28			POSITIVE	NT
77	CASE 85	00969	DNA	SPLEEN	20.43			POSITIVE	NT
78	CASE 85	00969	DNA	LYMPHNODE	20.9			POSITIVE	NT
79	CASE 85	00969	DNA	KIDNEY	21.84			POSITIVE	NT
80	CASE 85	00969	DNA	BLOOD-EDTA	19.1			POSITIVE	NT
81	CASE 87	04513	DNA	BLOOD-EDTA	19.87			POSITIVE	NT
82	CASE 89	05778	DNA	BONE MARROW	27.76			POSITIVE	NT
83	CASE 90	06985	DNA	BONE MARROW	29.35			POSITIVE	NT
84	CASE 91	07631	DNA	BONE MARROW	29.71			POSITIVE	NT
85	CASE 93	10375	DNA	BONE MARROW	24.72			POSITIVE	NT
86	CASE 94	12990	DNA	BONE MARROW	25.53			POSITIVE	NT
87	CASE 95	14055	DNA	BONE MARROW	18.29			POSITIVE	NT
88	CASE 95	14056	DNA	BONE MARROW	18.94			POSITIVE	NT
112	CASE 96	15777	DNA	BONE MARROW	27.21			POSITIVE	NT
113	CASE 96	15778	DNA	BONE MARROW	22.57			POSITIVE	NT



**Assays and activities marked with \* are not supported by ENAC accreditation.**

114	CASE 96	15779	DNA	BONE MARROW	25.77			POSITIVE	NT
115	CASE 96	15780	DNA	BONE MARROW	20.11			POSITIVE	NT
116	CASE 96	15780	DNA	SPLEEN	20.51			POSITIVE	NT
117	CASE 98	16080	DNA	BONE MARROW	20.76			POSITIVE	NT
118	CASE 99	16554	DNA	BONE MARROW	31.4			POSITIVE	NT
119	CASE 99	16555	DNA	SPLEEN	19.46			POSITIVE	NT
120	CASE 99	16555	DNA	KIDNEY	24.38			POSITIVE	NT
121	CASE 99	16557	DNA	KIDNEY	27.14			POSITIVE	NT
122	CASE 99	16557	DNA	SPLEEN	20.34			POSITIVE	NT
123	CASE 100	16565 - WB Nº7	DNA	BLOOD-EDTA	29.28			POSITIVE	NT
124	CASE 101	16677	DNA	BLOOD-EDTA	21.52			POSITIVE	NT
125	CASE 101	16677	DNA	KIDNEY	22.95			POSITIVE	NT
126	CASE 101	16677	DNA	SPLEEN	21.3			POSITIVE	NT
127	CASE 102	16858	DNA	BONE MARROW	24.42			POSITIVE	NT
128	CASE 103	17013	DNA	BONE MARROW	19.32			POSITIVE	NT
129	CASE 104	17014	DNA	BONE MARROW	19.97			POSITIVE	NT
130	CASE 104	17015	DNA	BONE MARROW	21.25			POSITIVE	NT
131	CASE 104	17016	DNA	BONE MARROW	28.9			POSITIVE	NT
132	CASE 105	17107	DNA	BONE MARROW	24.88			POSITIVE	NT

NT → Samples not tested. 1 → Result in 1/10 dilution.

(a) **UPL-real time PCR** → Real time PCR test described by Fernández-Pinero et al., 2013 based on the Universal Probe Library (UPL). [PNT/CISA/PPA/PCR/3]

(b) **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).

**1.2. DOMESTIC PIG SAMPLES** → **Positive ASFV result** has been obtained by the **UPL real-time PCR** in the 43 domestic pig samples tested, **confirming the presence of ASFV in all domestic pigs received**. After three passages in PAM, the ASFV has been isolated from 2 domestic pigs identified as **15553** (ID CISA 26) and **15555** (ID CISA 30) showing the classical haemadsorbing pattern of the ASFV circulating in Europe. The results obtained in virus detection are summarized in the **table 3**.

Sample ID CISA	Sample laboratory code in Poland's NRL				UPL-real time PCR <sup>(a)</sup>		VI-HAD <sup>(b)*</sup> (3 passages)
	ID tube		Sample Type	Ct value	Result		
15	OUTBREAK 4	15337	TISSUE KIDNEY	28.87	POSITIVE	NEG	
16	OUTBREAK 4	15337	TISSUE SPLEEN	27.53	POSITIVE	NEG	
17	OUTBREAK 4	15338	TISSUE KIDNEY	28.18	POSITIVE	NT	
18	OUTBREAK 4	15338	TISSUE SPLEEN	22.36	POSITIVE	NT	
19	OUTBREAK 4	15339	TISSUE KIDNEY	28.14	POSITIVE	NT	
20	OUTBREAK 4	15339	TISSUE SPLEEN	26.53	POSITIVE	NT	
21	OUTBREAK 4	15340	TISSUE KIDNEY	25.41	POSITIVE	NT	
22	OUTBREAK 4	15340	TISSUE SPLEEN	24.15	POSITIVE	NT	
23	OUTBREAK 4	15341	TISSUE KIDNEY	23.38	POSITIVE	NT	
24	OUTBREAK 4	15341	TISSUE SPLEEN	24.91	POSITIVE	NT	
25	OUTBREAK 4	15553	TISSUE KIDNEY	28.63	POSITIVE	NT	
26	OUTBREAK 4	15553	TISSUE SPLEEN	23.63	POSITIVE	POS	
27	OUTBREAK 4	15554	TISSUE KIDNEY	23.79	POSITIVE	NT	
28	OUTBREAK 4	15554	TISSUE SPLEEN	22.04	POSITIVE	NT	
29	OUTBREAK 4	15555	TISSUE KIDNEY	25.54	POSITIVE	NT	
30	OUTBREAK 4	15555	TISSUE SPLEEN	23.84	POSITIVE	POS	
31	OUTBREAK 4	15556	TISSUE KIDNEY	21.81	POSITIVE	NT	





**Assays and activities marked with \* are not supported by ENAC accreditation.**

32	OUTBREAK 4	15556	TISSUE	SPLEEN	22.01	POSITIVE	NT
64	OUTBREAK 4	15337	BLOOD	BLOOD-EDTA	21.74	POSITIVE	NT
65	OUTBREAK 4	15338	BLOOD	BLOOD-EDTA	23.51	POSITIVE	NT
66	OUTBREAK 4	15339	BLOOD	BLOOD-EDTA	NT	NT	NT
67	OUTBREAK 4	15340	BLOOD	BLOOD-EDTA	NT	NT	NT
68	OUTBREAK 4	15341	BLOOD	BLOOD-EDTA	NT	NT	NT
89	OUTBREAK 4	15337	DNA	BLOOD-EDTA	20.49	POSITIVE	NT
90	OUTBREAK 4	15337	DNA	KIDNEY	25.53	POSITIVE	NT
91	OUTBREAK 4	15337	DNA	SPLEEN	21.12	POSITIVE	NT
92	OUTBREAK 4	15338	DNA	BLOOD-EDTA	20.36	POSITIVE	NT
93	OUTBREAK 4	15338	DNA	KIDNEY	22	POSITIVE	NT
94	OUTBREAK 4	15338	DNA	SPLEEN	20.26	POSITIVE	NT
95	OUTBREAK 4	15339	DNA	BLOOD-EDTA	26.92	POSITIVE	NT
96	OUTBREAK 4	15339	DNA	KIDNEY	28.96	POSITIVE	NT
97	OUTBREAK 4	15339	DNA	SPLEEN	22.35	POSITIVE	NT
98	OUTBREAK 4	15340	DNA	BLOOD-EDTA	20.5	POSITIVE	NT
99	OUTBREAK 4	15340	DNA	KIDNEY	22.83	POSITIVE	NT
100	OUTBREAK 4	15340	DNA	SPLEEN	18.9	POSITIVE	NT
101	OUTBREAK 4	15341	DNA	BLOOD-EDTA	23.35	POSITIVE	NT
102	OUTBREAK 4	15341	DNA	KIDNEY	21.8	POSITIVE	NT
103	OUTBREAK 4	15341	DNA	SPLEEN	20.53	POSITIVE	NT
104	OUTBREAK 4	15553	DNA	KIDNEY	24.04	POSITIVE	NT
105	OUTBREAK 4	15553	DNA	SPLEEN	20.88	POSITIVE	NT
106	OUTBREAK 4	15554	DNA	KIDNEY	22.74	POSITIVE	NT
107	OUTBREAK 4	15554	DNA	SPLEEN	20.33	POSITIVE	NT
108	OUTBREAK 4	15555	DNA	KIDNEY	25.36	POSITIVE	NT
109	OUTBREAK 4	15555	DNA	SPLEEN	20.72	POSITIVE	NT
110	OUTBREAK 4	15556	DNA	KIDNEY	22.36	POSITIVE	NT
111	OUTBREAK 4	15556	DNA	SPLEEN	19.33	POSITIVE	NT

NT → Samples not tested.

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

[PNT/CISA/PPA/PCR/3]

b) **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).

## 2. ASF ANTIBODY DETECTION:

**2.1. WILD BOAR SAMPLES** → By IPT\*, the presence of antibodies was detected in 14 out of the 44 samples tested, which corresponds to **12 out of the 40 (30%)** wild boar received (table 4).

Sample ID CISA	Sample laboratory code		Sample Type		URL-IPT <sup>(a)</sup>		CONCLUSION*
	ID tube				Titer	Result	
1	CASE 75	19121/15	TISSUE	BONE MARROW	NEG	NEG	NEGATIVE
2	CASE 79	31071	TISSUE	BONE MARROW	NEG	NEG	NEGATIVE
3	CASE 80	32842	TISSUE	BONE MARROW	1/20	WEAK	WEAK
4	CASE 81	32981	TISSUE	BONE MARROW	NEG	NEG	NEGATIVE
5	CASE 85	00969	TISSUE	SPLEEN	NT	NT	NT
6	CASE 85	00969	TISSUE	LYMPHNODE	NT	NT	NT
7	CASE 85	00969	TISSUE	KIDNEY	NT	NT	NT
8	CASE 89	05778	TISSUE	BONE MARROW	NEG	NEG	NEGATIVE
9	CASE 90	06985	TISSUE	BONE MARROW	NEG	NEG	NEGATIVE
10	CASE 91	07631	TISSUE	BONE MARROW	NEG	NEG	NEGATIVE
11	CASE 93	10375	TISSUE	BONE MARROW	1/10	WEAK	WEAK



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12	CASE 94	12990	TISSUE	BONE MARROW	NEG	NEG	NEGATIVE
13	CASE 95	14055	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
14	CASE 95	14056	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
33	CASE 96	15777	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
34	CASE 96	15778	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
35	CASE 96	15779	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
36	CASE 96	15780	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
37	CASE 96	15780	TISSUE	SPLEEN	NEG	NEG	NEGATIVE
38	CASE 98	16080	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
39	CASE 99	16554	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
40	CASE 99	16555	TISSUE	SPLEEN	NEG	NEG	NEGATIVE
41	CASE 99	16555	TISSUE	KIDNEY	NEG	NEG	NEGATIVE
42	CASE 99	16557	TISSUE	SPLEEN	NEG	NEG	NEGATIVE
43	CASE 99	16557	TISSUE	KIDNEY	NT	NT	NT
44	CASE 101	16677	TISSUE	KIDNEY	1/320	POS	POSITIVE
45	CASE 101	16677	TISSUE	SPLEEN	1/160	POS	POSITIVE
46	CASE 102	16858	TISSUE	BONE MARROW	NEG	NEG	NEGATIVE
47	CASE 103	17013	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
48	CASE 104	17014	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
49	CASE 104	17015	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
50	CASE 104	17016	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
51	CASE 105	17107	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
52	CASE 42	05097	HOMOGENATE	BONE MARROW	NEG	NEG	NEGATIVE
53	CASE 76	19765 - WB Nº17	BLOOD	BLOOD-EDTA	1/320	POS	POSITIVE
54	CASE 77	27152 - WB Nº 55	BLOOD	BLOOD-EDTA	1/2560	POS	POSITIVE
55	CASE 78	30296 - WB Nº 10	BLOOD	BLOOD-EDTA	1/10240	POS	POSITIVE
56	CASE 83	33256 - WB Nº 22	BLOOD	BLOOD-EDTA	1/5120	POS	POSITIVE
57	CASE 82	33245 - WB Nº 3	BLOOD	BLOOD-EDTA	NEG	NEG	NEGATIVE
58	CASE 84	00347 - WB Nº 22	BLOOD	BLOOD-EDTA	NEG	NEG	NEGATIVE
59	CASE 85	00969	BLOOD	BLOOD-EDTA	NEG	NEG	NEGATIVE
60	CASE 86	03518 - WB Nº 8	BLOOD	BLOOD-EDTA	1/20480	POS	POSITIVE
61	CASE 87	04513	BLOOD	BLOOD-EDTA	NEG	NEG	NEGATIVE
62	CASE 88	05432 - WB Nº 12	BLOOD	BLOOD-EDTA	1/10240	POS	POSITIVE
63	CASE 92	08590 - WB Nº 1	BLOOD	BLOOD-EDTA	1/2560	POS	POSITIVE
69	CASE 97	15887 - WB Nº 3	BLOOD	BLOOD-EDTA	1/10240	POS	POSITIVE
70	CASE 100	16565 - WB Nº 7	BLOOD	BLOOD-EDTA	1/20480	POS	POSITIVE
71	CASE 101	16677	BLOOD	BLOOD-EDTA	1/1280	POS	POSITIVE

NT=NOT TESTED; WEAK= IPT Titre <1/80

- (a) URL-IPT → Indirect immunoperoxidase technique on E70-MS infected cells using the protocol standardized and validated by the European Union Reference Laboratory (EURL).

**2.2. DOMESTIC PIG SAMPLES** → By IPT\*, the presence of antibodies was detected in 19 out of the 22 samples tested which corresponds to **8 out of the 9 (88.8%)** domestic pig received (table 5).

Sample ID CISA	Sample laboratory code		URL-IPT* (a)		CONCLUSION*	
	ID tube	Sample Type	Titer	Result		
15	OUTBREAK 4	15337	TISSUE KIDNEY	1/80	POS	POSITIVE
16	OUTBREAK 4	15337	TISSUE SPLEEN	1/80	POS	POSITIVE
17	OUTBREAK 4	15338	TISSUE KIDNEY	NEG	NEG	NEGATIVE
18	OUTBREAK 4	15338	TISSUE SPLEEN	NEG	NEG	NEGATIVE
19	OUTBREAK 4	15339	TISSUE KIDNEY	1/2560	POS	POSITIVE
20	OUTBREAK 4	15339	TISSUE SPLEEN	1/1280	POS	POSITIVE
22	OUTBREAK 4	15340	TISSUE SPLEEN	1/80	POS	POSITIVE



**Assays and activities marked with \* are not supported by ENAC accreditation.**

23	OUTBREAK 4	15341	TISSUE	KIDNEY	1/320	POS	POSITIVE
24	OUTBREAK 4	15341	TISSUE	SPLEEN	1/160	POS	POSITIVE
25	OUTBREAK 4	15553	TISSUE	KIDNEY	1/80	POS	POSITIVE
26	OUTBREAK 4	15553	TISSUE	SPLEEN	1/80	POS	POSITIVE
27	OUTBREAK 4	15554	TISSUE	KIDNEY	1/640	POS	POSITIVE
28	OUTBREAK 4	15554	TISSUE	SPLEEN	1/1280	POS	POSITIVE
29	OUTBREAK 4	15555	TISSUE	KIDNEY	1/640	POS	POSITIVE
30	OUTBREAK 4	15555	TISSUE	SPLEEN	1/640	POS	POSITIVE
31	OUTBREAK 4	15556	TISSUE	KIDNEY	1/160	POS	POSITIVE
32	OUTBREAK 4	15556	TISSUE	SPLEEN	1/160	POS	POSITIVE
64	OUTBREAK 4	15337	BLOOD	BLOOD-EDTA	1/320	POS	POSITIVE
65	OUTBREAK 4	15338	BLOOD	BLOOD-EDTA	NEG	NEG	NEGATIVE
66	OUTBREAK 4	15339	BLOOD	BLOOD-EDTA	1/5120	POS	POSITIVE
67	OUTBREAK 4	15340	BLOOD	BLOOD-EDTA	1/320	POS	POSITIVE
68	OUTBREAK 4	15341	BLOOD	BLOOD-EDTA	1/1280	POS	POSITIVE

(a) URL-IPT → Indirect immunoperoxidase technique on E70-MS infected cells using the protocol standardized and validated by the European Union Reference Laboratory (EURL).

3. **ASFV MOLECULAR CHARACTERIZATION\*** → The expected ≈478 bp of the C-terminal p72-gene has been obtained in all samples selected (table 6). The PCR amplicons were sequenced in order to place the ASFV Poland viruses in one of the defined p72 genotypes. The wild boar ASFV Poland viruses clustered **within p72 genotype II** and showed 100% nucleotide identity with all compared ASFV isolates from Eastern and Central Europe across the 478-bp C-terminal p72 gene.

**Table 6 → Identification of the wild boar and domestic pigs ASFV sequenced**

ASFV SEQUENCED	HOST	SAMPLING LOCALIZATION	SAMPLING DATE	ID ANIMAL	ID CISA
Pol15/WB-CASE 42	Wild boar	Gródek	15/03/2015	05097/15	52
Pol15/WB-CASE 75	Wild boar	Białowieża	13/08/2015	19121	1
Pol15/WB-CASE 79	Wild boar	Narewka	27/11/2015	31071/15	2
Pol15/WB-CASE 80	Wild boar	Narewka	16/12/2015	32842/15	3
Pol15/WB-CASE 81	Wild boar	Białowieża	21/12/2015	32981/15	4
Pol16/WB-CASE 82	Wild boar	Narew	25/12/2015	33245-3/15	76
Pol16/WB-CASE 85	Wild boar	Narewka	20/01/2016	00969/16	59
Pol16/WB-CASE 87	Wild boar	Supraśl.	09/03/2016	0451/16	61
Pol16/WB-CASE 89	Wild boar	Narewka	22/03/2016	05778/16	8
Pol16/WB-CASE 90	Wild boar	Białowieża	11/04/2016	06985/16	9
Pol16/WB-CASE 91	Wild boar	Białowieża	15/04/2016	07631/16	10
Pol16/WB-CASE 93	Wild boar	Narewka	12/05/2016	10375/16	11
Pol16/WB-CASE 94	Wild boar	Narewka	03/06/2016	12990/16	12
Pol16/WB-CASE 95	Wild boar	Białowieża	14/06/2016	14055/16	13
Pol16/WB-CASE 97	Wild boar	Narewka	25/06/2016	15887-3/16	69
Pol16/WB-CASE 96	Wild boar	Czyże	26/06/2016	15780/16	37
Pol16/WB-CASE 98	Wild boar	Białowieża	29/06/2016	16080/16	38
Pol16/WB-CASE 100	Wild boar	Hajnówka	01/07/2016	16565-7/16	70
Pol16/WB-CASE 99	Wild boar	Hajnówka	04/07/2016	16555	40
Pol16/WB-CASE 101	Wild boar	Narewka	05/07/2016	16677	45
Pol16/WB-CASE 102	Wild boar	Hajnówka	06/07/2016	16858	46
Pol16/WB-CASE 103	Wild boar	Hajnówka	07/07/2016	17013	47
Pol16/WB-CASE 104	Wild boar	Hajnówka	08/07/2016	17014/16	48
Pol16/DP-OUT4 (16)	Domestic pig	Hajnówka	22/06/2016	15337/16	16
Pol16/DP-OUT4 (26)	Domestic pig	Hajnówka	23/06/2016	15553/16	26



*Assays and activities marked with \* are not supported by ENAC accreditation.*

Nucleotide and amino acid sequence analysis of the CVR-PCR product placed the Poland wild boar viruses within the **variant GII-CVR1** with the presence of 10 copies of amino acid tetramer repeats that were 100% identical and unique to those of the ASFV circulating in the Eastern countries since 2007.

Finally, the sequence analysis of the **intergenic region between the 173R and 1329L genes (IGR<sub>173R-1329L</sub>)** revealed the presence of the nucleotide tandem repeat (TRS) insertion (**GGAATATATA**) which is representative of all the ASFV isolates circulating within the EU countries Estonia, Latvia, Poland and Lithuania since the entrance of the disease in January 2014. This **intergenic region variant named GII-IGR<sub>173R-1329L</sub>-2** is also present in the Ukraine 2012 and 2015 isolates, in the Belarus 2013 viruses and, since 2012 in Russia Federation, mainly in the Russian Tulskeya region (Gallardo *et al.*, 2014; Goller *et al.*, 2015).

## CONCLUSION\*

1. The **presence of ASF** has been confirmed throughout antibody and/or ASFV genome detection in **all wild boar** received from the **cases 42 and from cases 75 to 105** which were occurred in **Poland from march 2015 (case 42) up to 9 July 2016 (case 105)**.
2. The **presence of ASF** has been confirmed in all **domestic pig** received from the fourth outbreak occurred on June 2016.
3. The **p72 genotyping of the new Poland wild boar and domestic pig ASFV** clustered the viruses within **p72 genotype II** circulating in the Eastern European countries since the first introduction in Georgia in 2007.
4. The **CVR subtyping** placed the ASF domestic pig and wild boar Poland viruses within the **CVR variant 1** identical to those of the ASFV circulating in the Eastern countries since 2007.
5. The **intergenic region (IGR 173R-1329L) subtyping** showed that the ASF Poland viruses responsible of the wild boar cases and domestic pig outbreaks were **100% homologous** to the viruses responsible of the cases occurred in wild boar and in domestic pigs in Lithuania, Latvia, Estonia and Poland since the ASFV entrance into the EU in 2014 and initially discovered in certain regions of the Russia Federation in 2012.



**Assays and activities marked with \* are not supported by ENAC accreditation.**

The table below summarizes the results obtained in each wild boar combining virus and antibody detection tests, as well as the genotyping results.

FINAL ASF DIAGNOSTIC RESULT IN WILD BOAR								
Sample ID CISA	OUTBREAK DATE	Sample laboratory code		VIRUS DETECTION	ANTIBODY DETECTION	ASFV	GENOTYPING (P72/CVR/IGR)	
1	13/08/2015	19121/15	CASE 75	Pogorzelle, Białowieża.	POSITIVE	NEGATIVE	Pol15/WB-CASE 75	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
2	27/11/2015	31071	CASE 79	Stoczek, Narewka.	POSITIVE	NEGATIVE	Pol15/WB-CASE 79	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
3	16/12/2015	32842	CASE 80	Olchowka, Narewka.	POSITIVE	WEAK <sup>(2)</sup>	Pol15/WB-CASE 80	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
4	21/12/2015	32981	CASE 81	Białowieża, Białowieża.	POSITIVE	NEGATIVE	Pol15/WB-CASE 81	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
5	20/01/2016	00969	CASE 85	Gruszki, Narewka.	POSITIVE	NEGATIVE	Pol16/WB-CASE 85	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
8	22/03/2016	05778	CASE 89	Zamosze/Gluzec, Narewka.	POSITIVE	NEGATIVE	Pol16/WB-CASE 89	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
9	11/04/2016	06985	CASE 90	Budy, Białowieża.	POSITIVE	NEGATIVE	Pol16/WB-CASE 90	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
10	15/04/2016	07631	CASE 91	Budy, Białowieża.	POSITIVE	NEGATIVE	Pol16/WB-CASE 91	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
11	12/05/2016	10375	CASE 93	Janowo, Narewka.	POSITIVE	WEAK	Pol16/WB-CASE 93	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
12	03/06/2016	12990	CASE 94	Krynica, Narewka.	POSITIVE	NEGATIVE	Pol16/WB-CASE 94	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
13	14/06/2016	14055	CASE 95	Teremiski, Białowieża.	POSITIVE	NEGATIVE	Pol16/WB-CASE 95	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
14	14/06/2016	14056	CASE 95	Teremiski, Białowieża.	POSITIVE	NEGATIVE		
33	26/06/2016	15777	CASE 96	Osovka, Czyże.	POSITIVE	NEGATIVE		
35	26/06/2016	15779	CASE 96	Osovka, Czyże.	POSITIVE	NEGATIVE		
36	26/06/2016	15780	CASE 96	Osovka, Czyże.	POSITIVE	NEGATIVE		
37	26/06/2016	15780	CASE 96	Osovka, Czyże.	POSITIVE	NEGATIVE	Pol16/WB-CASE 96	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
38	29/06/2016	16080	CASE 98	Podcerkwy, Białowieża.	POSITIVE	NEGATIVE	Pol16/WB-CASE 98	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
39	04/07/2016	16554	CASE 99	Sacharewo, Hajnówka.	POSITIVE	NEGATIVE		
40	04/07/2016	16555	CASE 99	Sacharewo, Hajnówka.	POSITIVE	NEGATIVE	Pol16/WB-CASE 99	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
42	04/07/2016	16557	CASE 99	Sacharewo, Hajnówka.	POSITIVE	NEGATIVE		
44	05/07/2016	16677	CASE 101	Tarnopol, Narewka.	POSITIVE	POSITIVE	Pol16/WB-CASE 101	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
46	06/07/2016	16858	CASE 102	Mochmate, Hajnówka.	POSITIVE	NEGATIVE	Pol16/WB-CASE 102	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
47	07/07/2016	17013	CASE 103	Lipiny, Hajnówka.	POSITIVE	NEGATIVE	Pol16/WB-CASE 103	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
48	08/07/2016	17014	CASE 104	Sacharewo, Hajnówka.	POSITIVE	NEGATIVE	Pol16/WB-CASE 104	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
49	08/07/2016	17015	CASE 104	Sacharewo, Hajnówka.	POSITIVE	NEGATIVE		
50	08/07/2016	17016	CASE 104	Sacharewo, Hajnówka.	POSITIVE	NEGATIVE		
51	09/07/2016	17107	CASE 105	Topilo, Hajnówka.	POSITIVE	NEGATIVE		
52	15/03/2015	05097	CASE 42	Bielewicz, Gródek.	POSITIVE	NEGATIVE	Pol15/WB-CASE 42	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
53	21/08/2015	19765-WB Nº 17	CASE 76	Lapicze, Krynki.	NEGATIVE	POSITIVE		
54	25/10/2015	27152-WB Nº 55	CASE 77	Straszewo, Gródek.	NEGATIVE	POSITIVE		
55	23/11/2015	30296-WB Nº 10	CASE 78	Pohorany, Sidra.	NEGATIVE	POSITIVE		
56	23/12/2015	33256-WB Nº 22	CASE 83	Mostowlany, Gródek.	NEGATIVE	POSITIVE		
57	25/12/2015	33245-WB Nº 3	CASE 82	Kaczaly, Narew.	POSITIVE	NEGATIVE	Pol16/WB-CASE 82	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
58	09/01/2016	00347-WB Nº 22	CASE 84	Waski, Narew.	WEAK <sup>(1)</sup>	NEGATIVE		
60	24/02/2016	03518-WB Nº 8	CASE 86	Bernacki Most, Narewka.	WEAK	POSITIVE		
61	09/03/2016	04513	CASE 87	Supraśl.	POSITIVE	NEGATIVE	Pol16/WB-CASE 87	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
62	17/03/2016	05432-WB Nº 12	CASE 88	Teremiski, Białowieża.	NEGATIVE	POSITIVE		
63	20/04/2016	08590-WB Nº 1	CASE 92	Kopce, Michałowo.	NEGATIVE	POSITIVE		
69	25/06/2016	15887-WB Nº 3	CASE 97	Bernacki Most, Narewka.	POSITIVE	POSITIVE	Pol16/WB-CASE 97	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2
70	01/07/2016	16565-WB Nº 7	CASE 100	Nieznaný Bor, Hajnówka.	POSITIVE	POSITIVE	Pol16/WB-CASE 100	GII/CVR-1/ IGR <sub>173R-1329L</sub> -2

(1) WEAK = PCR-UPL weak (Ct=35-40)

(2) WEAK = IPT weak titer < 1/80



**Assays and activities marked with \* are not supported by ENAC accreditation.**

The table below summarizes the results obtained in each domestic pig combining virus and antibody detection tests, as well as the genotyping results.

FINAL ASF DIAGNOSTIC RESULT IN DOMESTIC PIGS								
Sample ID CISA	OUTBREAK DATE	Sample laboratory code		VIRUS DETECTION	ANTIBODY DETECTION	ASFV	GENOTYPING (P72/CVR/IGR)	
16	22/06/2016	15337	OUTBREAK 4	Bielszczyszna, Hajnówka.	POSITIVE	POSITIVE	Pol16/DP-OUT4 <sup>(16)</sup>	GII/CVR- 1/ IGR <sub>I73R-1329L</sub> -2
17	22/06/2016	15338	OUTBREAK 4	Bielszczyszna, Hajnówka.	POSITIVE	NEGATIVE		GII/CVR- 1/ IGR <sub>I73R-1329L</sub> -2
19	22/06/2016	15339	OUTBREAK 4	Bielszczyszna, Hajnówka.	POSITIVE	POSITIVE		GII/CVR- 1/ IGR <sub>I73R-1329L</sub> -2
22	22/06/2016	15340	OUTBREAK 4	Bielszczyszna, Hajnówka.	POSITIVE	POSITIVE		GII/CVR- 1/ IGR <sub>I73R-1329L</sub> -2
23	22/06/2016	15341	OUTBREAK 4	Bielszczyszna, Hajnówka.	POSITIVE	POSITIVE		GII/CVR- 1/ IGR <sub>I73R-1329L</sub> -2
25	23/06/2016	15553	OUTBREAK 4	Bielszczyszna, Hajnówka.	POSITIVE	POSITIVE	Pol16/DP-OUT4 <sup>(26)</sup>	GII/CVR- 1/ IGR <sub>I73R-1329L</sub> -2
27	23/06/2016	15554	OUTBREAK 4	Bielszczyszna, Hajnówka.	POSITIVE	POSITIVE		GII/CVR- 1/ IGR <sub>I73R-1329L</sub> -2

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