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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: March 16th, 2021.

ARRIVAL DATE TO CISA: December 18th, 2020

RESPONSIBLE FOR SUBMISSION:

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Num. ARRIVAL REGISTER CISA: REG. 344/2020.

TEST REQUESTED: AFRICAN SWINE FEVER (ASF) CONFIRMATORY DIAGNOSIS

Num. SAMPLES RECEIVED: 179 samples from 84 wild boar (see table 1).

Sample ID CISA	Table 1 → Identification of the samples TESTED				TYPE SAMPLE RECEIVED	
	Date of sampling	ID animal	Locality	CASE		
1	12/03/2018	12860-3/18	Bartoszyce, Warminsko-mazurskie	1703	TISSUE	BONE MARROW
2	12/03/2018	12860-3/18	Bartoszyce, Warminsko-mazurskie	1703	HOMOGENATE	BONE MARROW
3	19/03/2018	14751-1/18	Bartoszyce, Warminsko-mazurskie	1746	TISSUE	BONE MARROW
4	19/03/2018	14751-1/18	Bartoszyce, Warminsko-mazurskie	1746	HOMOGENATE	BONE MARROW
5	19/03/2018	14751-6/18	Bartoszyce, Warminsko-mazurskie	1746	TISSUE	BONE MARROW
6	19/03/2018	14751-6/18	Bartoszyce, Warminsko-mazurskie	1746	HOMOGENATE	BONE MARROW
7	16/03/2018	14988-1/18	Bartoszyce, Warminsko-mazurskie	1774	TISSUE	BONE MARROW
8	16/03/2018	14988-1/18	Bartoszyce, Warminsko-mazurskie	1774	HOMOGENATE	BONE MARROW
9	16/03/2018	14988-2/18	Bartoszyce, Warminsko-mazurskie	1774	TISSUE	BONE MARROW
10	16/03/2018	14988-2/18	Bartoszyce, Warminsko-mazurskie	1774	HOMOGENATE	BONE MARROW
11	16/03/2018	14988-4/18	Bartoszyce, Warminsko-mazurskie	1774	TISSUE	BONE MARROW
12	16/03/2018	14988-4/18	Bartoszyce, Warminsko-mazurskie	1774	HOMOGENATE	BONE MARROW
13	16/03/2018	14988-5/18	Bartoszyce, Warminsko-mazurskie	1774	TISSUE	BONE MARROW
14	16/03/2018	14988-5/18	Bartoszyce, Warminsko-mazurskie	1774	HOMOGENATE	BONE MARROW
15	16/03/2018	14988-7/18	Bartoszyce, Warminsko-mazurskie	1774	TISSUE	BONE MARROW
16	16/03/2018	14988-7/18	Bartoszyce, Warminsko-mazurskie	1774	HOMOGENATE	BONE MARROW
17	16/03/2018	14988-8/18	Bartoszyce, Warminsko-mazurskie	1774	TISSUE	BONE MARROW
18	16/03/2018	14988-8/18	Bartoszyce, Warminsko-mazurskie	1774	HOMOGENATE	BONE MARROW
19	19/03/2018	15286-3/18	Nasielsk, Mazowieckie	1775	HOMOGENATE	BONE MARROW



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Sample ID CISA	Sample laboratory code ¹				TYPE SAMPLE RECEIVED	
	Date of sampling	ID animal	Locality	CASE		
20	21/03/2018	15390-1/18	Gorowo llawieckie, Warminsko-mazurskie	1793	TISSUE	BONE MARROW
21	21/03/2018	15390-1/18	Gorowo llawieckie, Warminsko-mazurskie	1793	HOMOGENATE	BONE MARROW
22	21/03/2018	15390-2/18	Gorowo llawieckie, Warminsko-mazurskie	1794	TISSUE	BONE MARROW
23	21/03/2018	15390-2/18	Gorowo llawieckie, Warminsko-mazurskie	1794	HOMOGENATE	BONE MARROW
24	21/03/2018	15390-3/18	Gorowo llawieckie, Warminsko-mazurskie	1794	TISSUE	BONE MARROW
25	21/03/2018	15390-3/18	Gorowo llawieckie, Warminsko-mazurskie	1794	HOMOGENATE	BONE MARROW
26	21/03/2018	15392/18	Bartoszyce, Warminsko-mazurskie	1795	TISSUE	BONE MARROW
27	21/03/2018	15392/18	Bartoszyce, Warminsko-mazurskie	1795	HOMOGENATE	BONE MARROW
28	03/04/2018	17567-2/18	Zmudz, Lubelskie	1865	TISSUE	BONE MARROW
29	03/04/2018	17567-2/18	Zmudz, Lubelskie	1865	HOMOGENATE	BONE MARROW
30	03/04/2018	17567-3/18	Zmudz, Lubelskie	1865	TISSUE	BONE MARROW
31	03/04/2018	17567-3/18	Zmudz, Lubelskie	1865	HOMOGENATE	BONE MARROW
32	03/04/2018	17561/8	Zmudz, Lubelskie	1865	TISSUE	SPLEEN
33	03/04/2018	17561/8	Zmudz, Lubelskie	1865	HOMOGENATE	SPLEEN
34	03/07/2018	38656/18	Banie Mazurskie, Warminsko-mazurskie	2458	TISSUE	BONE MARROW
35	03/07/2018	38656/18	Banie Mazurskie, Warminsko-mazurskie	2458	HOMOGENATE	BONE MARROW
36	12/07/2018	40176-1/18	Dolhobyczow, Lubelskie	2542	HOMOGENATE	BONE MARROW
37	15/07/2018	40586-1/18	Ostrow Lubelski, Lubelskie	2543	HOMOGENATE	BONE MARROW
38	12/07/2018	40663-1/18	Grabow nad Pilica, Mazowieckie	2548	TISSUE	BONE MARROW
39	12/07/2018	40663-1/18	Grabow nad Pilica, Mazowieckie	2548	HOMOGENATE	BONE MARROW
40	18/07/2018	41877/18	Srokowo, Warminsko-mazurskie	2574	HOMOGENATE	BONE MARROW
41	14/07/2018	41879-2/18	Barciany, Warminsko-mazurskie	2576	TISSUE	BONE MARROW
42	14/07/2018	41879-2/18	Barciany, Warminsko-mazurskie	2576	HOMOGENATE	BONE MARROW
43	23/08/2018	50033/18	Tolkmicko, Warminsko-mazurskie	2766	TISSUE	BONE MARROW
44	23/08/2018	50033/18	Tolkmicko, Warminsko-mazurskie	2766	HOMOGENATE	BONE MARROW
45	30/08/2018	51617/18	Korsze, Warminsko-mazurskie	2778	HOMOGENATE	BONE MARROW
46	06/09/2018	53500-1/18	Tolkmicko, Warminsko-mazurskie	2819	TISSUE	BONE MARROW
47	06/09/2018	53500-1/18	Tolkmicko, Warminsko-mazurskie	2819	HOMOGENATE	BONE MARROW
48	06/09/2018	53500-2/18	Tolkmicko, Warminsko-mazurskie	2819	TISSUE	BONE MARROW
49	06/09/2018	53500-2/18	Tolkmicko, Warminsko-mazurskie	2819	HOMOGENATE	BONE MARROW
50	03/09/2018	52838-2/18	Tolkmicko, Warminsko-mazurskie	2820	TISSUE	BONE MARROW
51	03/09/2018	52838-2/18	Tolkmicko, Warminsko-mazurskie	2820	HOMOGENATE	BONE MARROW
52	13/09/2018	55233-1/18	Ketrzyn, Warminsko-mazurskie	2839	TISSUE	BONE MARROW
53	13/09/2018	55233-1/18	Ketrzyn, Warminsko-mazurskie	2839	HOMOGENATE	BONE MARROW
54	13/09/2018	55233-3/18	Ketrzyn, Warminsko-mazurskie	2839	TISSUE	BONE MARROW
55	13/09/2018	55233-3/18	Ketrzyn, Warminsko-mazurskie	2839	HOMOGENATE	BONE MARROW
56	13/09/2018	55233-4/18	Ketrzyn, Warminsko-mazurskie	2839	TISSUE	BONE MARROW
57	13/09/2018	55233-4/18	Ketrzyn, Warminsko-mazurskie	2839	HOMOGENATE	BONE MARROW
58	13/09/2018	56247/18	Lidzbark Warminski, Warminsko-mazurskie	2850	TISSUE	BONE MARROW
59	13/09/2018	56247/18	Lidzbark Warminski, Warminsko-mazurskie	2850	HOMOGENATE	BONE MARROW
60	13/12/2018	75885-1/18	Wydmyny, Warminsko-mazurskie	3314	TISSUE	BONE MARROW
61	13/12/2018	75885-1/18	Wydmyny, Warminsko-mazurskie	3314	HOMOGENATE	BONE MARROW



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62	13/12/2018	75885-2/18	Wydminy, Warminsko-mazurskie	3314	TISSUE	BONE MARROW
63	13/12/2018	75885-2/18	Wydminy, Warminsko-mazurskie	3314	HOMOGENATE	BONE MARROW
64	17/12/2018	76038/18	Paslek, Warminsko-mazurskie	3315	TISSUE	BONE MARROW
65	17/12/2018	76038/18	Paslek, Warminsko-mazurskie	3315	HOMOGENATE	BONE MARROW
66	17/12/2018	76040/18	Gorzno, Kujawsko-pomorskie	3310	TISSUE	BONE MARROW
67	17/12/2018	76040/18	Gorzno, Kujawsko-pomorskie	3310	HOMOGENATE	BONE MARROW
68	22/03/2018	16235-1/18	Dabrowka, Mazowieckie	1836	BLOOD	SERUM
69	07/05/2018	24513-1/18	Bielsk Podlaski, Podlaskie	2100	BLOOD	SERUM
70	10/05/2018	26020-2/18	Nadarzyn, Mazowieckie	2136	BLOOD	SERUM
71	14/05/2018	26376-4/18	Dorohusk, Lubelskie	2155	BLOOD	SERUM
72	23/05/2018	29838-11/18	Olecko, Warminsko-mazurskie	2198	BLOOD	SERUM
73	18/12/2018	75806-2/18	Stezyca, Lubelskie	3276	BLOOD	SERUM
74	18/06/2020	29267/19	Tomaszow Lubelski, Lubelskie	1298	HOMOGENATE	SPLEEN
75	18/06/2020	29267/19	Tomaszow Lubelski, Lubelskie	1298	HOMOGENATE	LYMPH NODE
76	18/06/2020	29267/19	Tomaszow Lubelski, Lubelskie	1298	HOMOGENATE	KIDNEY
77	18/06/2020	29267/19	Tomaszow Lubelski, Lubelskie	1298	HOMOGENATE	LUNG
78	03/07/2019	32881/19	Ciechanow, Mazowieckie	1383	HOMOGENATE	BONE MARROW
79	03/07/2019	32881/19	Ciechanow, Mazowieckie	1383	HOMOGENATE	SPLEEN
80	03/07/2019	32881/19	Ciechanow, Mazowieckie	1383	HOMOGENATE	KIDNEY
81	18/07/2019	34250/19	Ciechanow, Mazowieckie	1436	HOMOGENATE	KIDNEY
82	18/07/2019	34251/19	Ciechanow, Mazowieckie	1436	HOMOGENATE	BONE MARROW
83	20/07/2019	35786/19	Ciechanow, Mazowieckie	1506	HOMOGENATE	BONE MARROW
84	29/07/2019	36159-1/19	Sulow, Lubelskie	1502	HOMOGENATE	BONE MARROW
85	02/08/2019	36938-1	Tarnograd, Lubelskie	1526	HOMOGENATE	BONE MARROW
86	02/08/2019	36938-2	Tarnograd, Lubelskie	1526	HOMOGENATE	BONE MARROW
87	02/08/2019	36938-3	Tarnograd, Lubelskie	1526	HOMOGENATE	BONE MARROW
88	07/08/2019	37465-2/19	Tarnograd, Lubelskie	1541	HOMOGENATE	BONE MARROW
89	07/08/2019	37465-3/19	Tarnograd, Lubelskie	1541	HOMOGENATE	BONE MARROW
90	13/08/2019	38346/19	Nasielsk, Mazowieckie	1589	TISSUE	BONE MARROW
91	13/08/2019	38346/19	Nasielsk, Mazowieckie	1589	HOMOGENATE	BONE MARROW
92	22/09/2019	51469/19	Orneta, Warminsko-mazurskie	1912	TISSUE	BONE MARROW
93	22/09/2019	51469/19	Orneta, Warminsko-mazurskie	1912	HOMOGENATE	BONE MARROW
94	16/11/2019	54186/19	Nowa Sol, Lubuskie	1973	TISSUE	LIVER
95	16/11/2019	54186/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	LIVER
96	16/11/2019	54188/19	Nowa Sol, Lubuskie	1973	TISSUE	BONE MARROW
97	16/11/2019	54188/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	BONE MARROW
98	16/11/2019	54188/19	Nowa Sol, Lubuskie	1973	TISSUE	SPLEEN
99	16/11/2019	54188/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	SPLEEN
100	16/11/2019	54188/19	Nowa Sol, Lubuskie	1973	TISSUE	LUNG
101	16/11/2019	54188/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	LUNG
102	16/11/2019	54188/19	Nowa Sol, Lubuskie	1973	TISSUE	LIVER
103	16/11/2019	54188/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	LIVER
104	16/11/2019	54190/19	Nowa Sol, Lubuskie	1973	TISSUE	SPLEEN
105	16/11/2019	54190/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	SPLEEN
106	16/11/2019	54190/19	Nowa Sol, Lubuskie	1973	TISSUE	LUNG
107	16/11/2019	54190/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	LUNG
108	16/11/2019	54190/19	Nowa Sol, Lubuskie	1973	TISSUE	LIVER
109	16/11/2019	54190/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	LIVER
110	16/11/2019	54192/19	Nowa Sol, Lubuskie	1973	TISSUE	BONE MARROW
111	16/11/2019	54192/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	BONE MARROW
112	16/11/2019	54192/19	Nowa Sol, Lubuskie	1973	TISSUE	SPLEEN



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Sample ID CISA	Sample laboratory code ¹				TYPE SAMPLE RECEIVED	
	Date of sampling	ID animal	Locality	CASE		
113	16/11/2019	54192/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	SPLEEN
114	16/11/2019	54192/19	Nowa Sol, Lubuskie	1973	TISSUE	LIVER
115	16/11/2019	54192/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	LIVER
116	16/11/2019	54194/19	Nowa Sol, Lubuskie	1973	TISSUE	BONE MARROW
117	16/11/2019	54194/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	BONE MARROW
118	16/11/2019	54194/19	Nowa Sol, Lubuskie	1973	TISSUE	SPLEEN
119	16/11/2019	54194/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	SPLEEN
120	16/11/2019	54194/19	Nowa Sol, Lubuskie	1973	TISSUE	LUNG
121	16/11/2019	54194/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	LUNG
122	16/11/2019	54194/19	Nowa Sol, Lubuskie	1973	TISSUE	LIVER
123	16/11/2019	54194/19	Nowa Sol, Lubuskie	1973	HOMOGENATE	LIVER
124	17/11/2019	54198/19	Nowa Sol, Lubuskie	1976	TISSUE	BONE MARROW
125	17/11/2019	54198/19	Nowa Sol, Lubuskie	1976	HOMOGENATE	BONE MARROW
126	17/11/2019	54198/19	Nowa Sol, Lubuskie	1976	TISSUE	SPLEEN
127	17/11/2019	54198/19	Nowa Sol, Lubuskie	1976	HOMOGENATE	SPLEEN
128	17/11/2019	54198/19	Nowa Sol, Lubuskie	1976	TISSUE	LUNG
129	17/11/2019	54198/19	Nowa Sol, Lubuskie	1976	HOMOGENATE	LUNG
130	17/11/2019	54202/19	Nowa Sol, Lubuskie	1977	TISSUE	BONE MARROW
131	17/11/2019	54202/19	Nowa Sol, Lubuskie	1977	HOMOGENATE	BONE MARROW
132	17/11/2019	54202/19	Nowa Sol, Lubuskie	1977	HOMOGENATE	SPLEEN
133	17/11/2019	54204/19	Nowa Sol, Lubuskie	1977	TISSUE	BONE MARROW
134	17/11/2019	54204/19	Nowa Sol, Lubuskie	1977	HOMOGENATE	BONE MARROW
135	17/11/2019	54204/19	Nowa Sol, Lubuskie	1977	TISSUE	SPLEEN
136	17/11/2019	54204/19	Nowa Sol, Lubuskie	1977	HOMOGENATE	SPLEEN
137	17/11/2019	54204/19	Nowa Sol, Lubuskie	1977	TISSUE	KIDNEY
138	17/11/2019	54204/19	Nowa Sol, Lubuskie	1977	HOMOGENATE	KIDNEY
139	17/11/2019	54204/19	Nowa Sol, Lubuskie	1977	TISSUE	LUNG
140	17/11/2019	54204/19	Nowa Sol, Lubuskie	1977	HOMOGENATE	LUNG
141	17/11/2019	54206/19	Nowa Sol, Lubuskie	1977	TISSUE	LUNG
142	17/11/2019	54206/19	Nowa Sol, Lubuskie	1977	HOMOGENATE	LUNG
143	17/11/2019	54208/19	Nowa Sol, Lubuskie	1977	TISSUE	BONE MARROW
144	17/11/2019	54208/19	Nowa Sol, Lubuskie	1977	HOMOGENATE	BONE MARROW
145	17/11/2019	54208/19	Nowa Sol, Lubuskie	1977	TISSUE	SPLEEN
146	17/11/2019	54208/19	Nowa Sol, Lubuskie	1977	HOMOGENATE	SPLEEN
147	17/11/2019	54210/19	Kolsko, Lubuskie	1978	TISSUE	LUNG
148	17/11/2019	54210/19	Kolsko, Lubuskie	1978	HOMOGENATE	LUNG
149	17/11/2019	54212/19	Kolsko, Lubuskie	1978	TISSUE	BONE MARROW
150	17/11/2019	54212/19	Kolsko, Lubuskie	1978	HOMOGENATE	BONE MARROW
151	17/11/2019	54212/19	Kolsko, Lubuskie	1978	TISSUE	SPLEEN
152	17/11/2019	54212/19	Kolsko, Lubuskie	1978	HOMOGENATE	SPLEEN
153	17/11/2019	54212/19	Kolsko, Lubuskie	1978	TISSUE	KIDNEY
154	17/11/2019	54212/19	Kolsko, Lubuskie	1978	HOMOGENATE	KIDNEY
155	17/11/2019	54212/19	Kolsko, Lubuskie	1978	TISSUE	LUNG
156	17/11/2019	54212/19	Kolsko, Lubuskie	1978	HOMOGENATE	LUNG
157	16/11/2019	54214-5/19	Kolsko, Lubuskie	1979	TISSUE	BONE MARROW
158	16/11/2019	54214-5/19	Kolsko, Lubuskie	1979	HOMOGENATE	BONE MARROW
159	25/11/2019	56264-2/19	Krzeszow, Podkarpackie	2108	BLOOD	SERUM
160	25/11/2019	56264-3/19	Krzeszow, Podkarpackie	2109	BLOOD	SERUM
161	25/11/2019	56265-1/19	Krzeszow, Podkarpackie	2106	TISSUE	BONE MARROW
162	25/11/2019	56265-1/19	Krzeszow, Podkarpackie	2106	HOMOGENATE	BONE MARROW
163	25/11/2019	56265-2/19	Krzeszow, Podkarpackie	2106	TISSUE	BONE MARROW



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	Date of sampling	ID animal	Locality	CASE		
164	25/11/2019	56265-2/19	Krzeszow, Podkarpacie	2106	HOMOGENATE	BONE MARROW
165	25/11/2019	56265-3/19	Krzeszow, Podkarpacie	2106	TISSUE	BONE MARROW
166	25/11/2019	56265-3/19	Krzeszow, Podkarpacie	2106	HOMOGENATE	BONE MARROW
167	26/11/2019	56941-2/19	Krzeszow, Podkarpacie	2137	TISSUE	BONE MARROW
168	26/11/2019	56941-2/19	Krzeszow, Podkarpacie	2137	HOMOGENATE	BONE MARROW
169		59071-1/19	Wolszyn, Wielkopolskie	2219	HOMOGENATE	BONE MARROW
170		59071-2/19	Wolszyn, Wielkopolskie	2219	HOMOGENATE	BONE MARROW
171		59071-3/19	Wolszyn, Wielkopolskie	2219	HOMOGENATE	BONE MARROW
172		59071-5/19	Wolszyn, Wielkopolskie	2219	HOMOGENATE	BONE MARROW
173		59071-6/19	Wolszyn, Wielkopolskie	2219	HOMOGENATE	BONE MARROW
174		59071-7/19	Wolszyn, Wielkopolskie	2219	HOMOGENATE	BONE MARROW
175		59071-8/19	Wolszyn, Wielkopolskie	2219	HOMOGENATE	BONE MARROW
176		59071-9/19	Wolszyn, Wielkopolskie	2219	HOMOGENATE	BONE MARROW
177		59071-11/19	Wolszyn, Wielkopolskie	2219	HOMOGENATE	BONE MARROW
178	11/12/2019	60342-1/19	Wolszyn, Wielkopolskie	2309	TISSUE	BONE MARROW
179	11/12/2019	60342-1/19	Wolszyn, Wielkopolskie	2309	HOMOGENATE	BONE MARROW

*INIA-CISA is not responsible for the identification/information of the samples provided by the client.

EXECUTION DATE: From 5th January to 1st March 2021.

ASF DIAGNOSTIC TESTS PERFORMED:

1. ASF virus detection.

1.1. ASFV genome detection → 10% (w/v) clarified homogenized tissue's suspensions have been prepared in phosphate-buffered saline (PBS) from 70 tissues [PNT/CISA/PPA/MUESTRAS/1]. The DNA was extracted from these **70 tissue homogenates**, from **32 homogenates** prepared at the Poland's NRL and from **8 serum samples**, 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 genomic DNA the UPL real-time PCR (Fernández-Pinero *et al.*, 2013; OIE 2019) [PNT/CISA/PPA/PCR/3] was carried out using the undiluted extracted DNA at the EURL in a total of **110 samples**. **Sixty nine (69) samples were not analysed since correspond to samples previously processed at the NRL (homogenates) from which original tissue was received at the EURL.**

1.2. ASF virus isolation and haemadsorption (HAD) assay* [PNT/CISA/PPA/VI/1] has been done on porcine blood monocytes (PBM) according is described in the OIE Manual (OIE 2019). The PBM has been inoculated at a multiplicity of infection (moi) 1:10 with **42 PCR positive samples** (8 wells/per sample; 10 µl inoculum per well). Samples have been filtered and treated with antibiotic (gentamicin sulphate) [PNT/CISA/PPA/MUESTRAS/1]. After inoculation, a



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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% CO₂, at 37°C. The plate are examined for haemadsorption over a period of seven days.

2. **ASF serological diagnosis** → For ASF antibody detection, the 8 serum samples, 70 tissues and 32 homogenates prepared at the Poland's NRL were analysed using the IPT [PNT/CISA/PPA/IPT/1] at 1/40 (exudates from tissues and serum samples) or from 1/5 (homogenates). Positive IPT results were titrated in two fold dilutions starting from 1/20 and 1/5, respectively, by the IPT. The 8 sera were also tested by the commercial blocking ELISA (INGEZIM PPA COMPAC, K3 INGENASA) [PNT/CISA/PPA/ELISA/2], and if doubts or positives, with the Immunoblotting test [PNT/CISA/PPA/IB/1]
3. **ASFV molecular characterization*** → Genetic characterization of ASFV has been performed from all PCR positive samples by PCR throughout the analysis of three variable regions of the ASFV genome which comprises: i) the intergenic region located between the *I73R* and *I329L* genes and characterized by the presence of tandem repeat sequences (TRS) (Gallardo *et al.*, 2014), ii) the central variable region (CVR) within the *B602L*-gene (Gallardo *et al.*, 2011) and iii) the intergenic region between the multigene family (MGF) 505 9R and the 10R genes of ASFV genome (Elsukova, A., *et al.*, 2016).

The nucleotide sequences obtained were compared with **2,116 representative genotype II ASFVs isolated since April 2007 up to February 2020 from wild and domestic pigs in Eastern and Central European countries** (Belgium, Bulgaria, Greece, Romania, Hungary, Czech Republic, Lithuania, Poland, Latvia, Estonia, Serbia, Slovakia, Belarus, Ukraine, Armenia, Azerbaijan, Georgia, Moldova and Russia Federation) available at the EURL. In addition were compared with the ASFV Germany 2020/1 isolate (Genbank Acc Nº LR899193.1) sequenced at the German's NRL.

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RESULTS.

1. **ASF virus detection** → **103** of the 110 samples tested have been **positive by PCR** (samples ID CISA 68, 69, 71, 74, 76, 77, 78, 79, 80, 88, 89, 159, 160, 170, 172 and 173 have been tested at 1/10 dilution). After three passages on PBMs, the virus could not be isolated in any of the 42-PCR positive samples included on virus isolation (**table 2**).

Table 2→ VIRUS DETECTION RESULTS						
Sample ID CISA	ID POLAND'S NRL		UPL-real time PCR ^(a)		VI-HAD ^{(b)*} 3rd PASSAGE	
	ID animal	TYPE OF SAMPLE RECEIVED	CT value	Result		
1	12860-3/18	TISSUE BONE MARROW	29.64	POSITIVE	NEGATIVE	
3	14751-1/18	TISSUE BONE MARROW	27.06	POSITIVE	NEGATIVE	
5	14751-6/18	TISSUE BONE MARROW	27.64	POSITIVE	NT	
7	14988-1/18	TISSUE BONE MARROW	20.99	POSITIVE	NEGATIVE	
9	14988-2/18	TISSUE BONE MARROW	27.06	POSITIVE	NT	
11	14988-4/18	TISSUE BONE MARROW	33.36	POSITIVE	NT	
13	14988-5/18	TISSUE BONE MARROW	32.55	POSITIVE	NT	
15	14988-7/18	TISSUE BONE MARROW	27.79	POSITIVE	NT	
17	14988-8/18	TISSUE BONE MARROW	24.69	POSITIVE	NT	
19	15286-3/18	HOMOGENATE BONE MARROW	32.81	POSITIVE	NEGATIVE	
20	15390-1/18	TISSUE BONE MARROW	23.88	POSITIVE	NEGATIVE	
22	15390-2/18	TISSUE BONE MARROW	22.21	POSITIVE	NEGATIVE	
24	15390-3/18	TISSUE BONE MARROW	34.32	POSITIVE	NT	
26	15392/18	TISSUE BONE MARROW	27.83	POSITIVE	NEGATIVE	
28	17567-2/18	TISSUE BONE MARROW	28.12	POSITIVE	NT	
30	17567-3/18	TISSUE BONE MARROW	23.16	POSITIVE	NEGATIVE	
32	17561/8	TISSUE SPLEEN	31.00	POSITIVE	NT	
34	38656/18	TISSUE BONE MARROW	29.42	POSITIVE	NEGATIVE	
36	40176-1/18	HOMOGENATE BONE MARROW	25.89	POSITIVE	NEGATIVE	
37	40586-1/18	HOMOGENATE BONE MARROW	27.48	POSITIVE	NEGATIVE	
38	40663-1/18	TISSUE BONE MARROW	30.7	POSITIVE	NEGATIVE	
40	41877/18	HOMOGENATE BONE MARROW	29.33	POSITIVE	NEGATIVE	
41	41879-2/18	TISSUE BONE MARROW	No Ct	NEGATIVE	NT	
43	50033/18	TISSUE BONE MARROW	23.76	POSITIVE	NEGATIVE	
45	51617/18	HOMOGENATE BONE MARROW	23.84	POSITIVE	NEGATIVE	
46	53500-1/18	TISSUE BONE MARROW	20.88	POSITIVE	NT	
48	53500-2/18	TISSUE BONE MARROW	20.7	POSITIVE	NEGATIVE	
50	52838-2/18	TISSUE BONE MARROW	20.75	POSITIVE	NEGATIVE	
52	55233-1/18	TISSUE BONE MARROW	29.34	POSITIVE	NT	
54	55233-3/18	TISSUE BONE MARROW	27.91	POSITIVE	NEGATIVE	
56	55233-4/18	TISSUE BONE MARROW	33.54	POSITIVE	NT	
58	56247/18	TISSUE BONE MARROW	26.78	POSITIVE	NEGATIVE	
60	75885-1/18	TISSUE BONE MARROW	23.73	POSITIVE	NEGATIVE	
62	75885-2/18	TISSUE BONE MARROW	24.99	POSITIVE	NT	
64	76038/18	TISSUE BONE MARROW	23.29	POSITIVE	NEGATIVE	



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Sample ID CISA	ID POLAND'S NRL		UPL-real time PCR ^(a)		VI-HAD ^{(b)*} 3rd PASSAGE
	ID animal	TYPE OF SAMPLE RECEIVED	CT value	Result	
66	76040/18	TISSUE BONE MARROW	23.24	POSITIVE	NEGATIVE
68	16235-1/18	BLOOD SERUM	No Ct	NEGATIVE	NT
69	24513-1/18	BLOOD SERUM	No Ct	NEGATIVE	NT
70	26020-2/18	BLOOD SERUM	No Ct	NEGATIVE	NT
71	26376-4/18	BLOOD SERUM	No Ct	NEGATIVE	NT
72	29838-11/18	BLOOD SERUM	No Ct	NEGATIVE	NT
73	75806-2/18	BLOOD SERUM	No Ct	NEGATIVE	NT
74	29267/19	HOMOGENATE SPLEEN	29.06	POSITIVE	NEGATIVE
75	29267/19	HOMOGENATE LYMPH NODE	33.26	POSITIVE	NT
76	29267/19	HOMOGENATE KIDNEY	34.62	POSITIVE	NT
77	29267/19	HOMOGENATE LUNG	31.49	POSITIVE	NT
78	32881/19	HOMOGENATE BONE MARROW	21.18	POSITIVE	NT
79	32881/19	HOMOGENATE SPLEEN	20.6	POSITIVE	NEGATIVE
80	32881/19	HOMOGENATE KIDNEY	27.00	POSITIVE	NT
81	34250/19	HOMOGENATE KIDNEY	27.51	POSITIVE	NT
82	34251/19	HOMOGENATE BONE MARROW	25.51	POSITIVE	NEGATIVE
83	35786/19	HOMOGENATE BONE MARROW	24.89	POSITIVE	NEGATIVE
84	36159-1/19	HOMOGENATE BONE MARROW	26.27	POSITIVE	NEGATIVE
85	36938-1	HOMOGENATE BONE MARROW	33.5	POSITIVE	NT
86	36938-2	HOMOGENATE BONE MARROW	31.13	POSITIVE	NT
87	36938-3	HOMOGENATE BONE MARROW	26.7	POSITIVE	NEGATIVE
88	37465-2/19	HOMOGENATE BONE MARROW	33.84	POSITIVE	NT
89	37465-3/19	HOMOGENATE BONE MARROW	26.98	POSITIVE	NEGATIVE
90	38346/19	TISSUE BONE MARROW	26.61	POSITIVE	NEGATIVE
92	51469/19	TISSUE BONE MARROW	30.84	POSITIVE	NEGATIVE
94	54186/19	TISSUE LIVER	27.7	POSITIVE	NT
96	54188/19	TISSUE BONE MARROW	28.31	POSITIVE	NT
98	54188/19	TISSUE SPLEEN	28.9	POSITIVE	NT
100	54188/19	TISSUE LUNG	26.24	POSITIVE	NT
102	54188/19	TISSUE LIVER	25.85	POSITIVE	NT
104	54190/19	TISSUE SPLEEN	28.99	POSITIVE	NT
106	54190/19	TISSUE LUNG	27.6	POSITIVE	NT
108	54190/19	TISSUE LIVER	25.9	POSITIVE	NT
110	54192/19	TISSUE BONE MARROW	28.25	POSITIVE	NT
112	54192/19	TISSUE SPLEEN	23.41	POSITIVE	NEGATIVE
113	54192/19	HOMOGENATE SPLEEN	25.89	POSITIVE	NT
114	54192/19	TISSUE LIVER	25.33	POSITIVE	NT
116	54194/19	TISSUE BONE MARROW	26.95	POSITIVE	NT
118	54194/19	TISSUE SPLEEN	28.36	POSITIVE	NT
120	54194/19	TISSUE LUNG	24.75	POSITIVE	NT
122	54194/19	TISSUE LIVER	20.81	POSITIVE	NT
124	54198/19	TISSUE BONE MARROW	34.13	POSITIVE	NT
126	54198/19	TISSUE SPLEEN	21.97	POSITIVE	NEGATIVE
128	54198/19	TISSUE LUNG	22.35	POSITIVE	NT



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Sample ID CISA	ID POLAND'S NRL		UPL-real time PCR ^(a)		VI-HAD ^{(b)*} 3rd PASSAGE
	ID animal	TYPE OF SAMPLE RECEIVED	CT value	Result	
130	54202/19	TISSUE BONE MARROW	27.62	POSITIVE	NT
132	54202/19	HOMOGENATE SPLEEN	28.69	POSITIVE	NT
133	54204/19	TISSUE BONE MARROW	27.2	POSITIVE	NT
135	54204/19	TISSUE SPLEEN	23.79	POSITIVE	NEGATIVE
137	54204/19	TISSUE KIDNEY	26.63	POSITIVE	NT
139	54204/19	TISSUE LUNG	24.87	POSITIVE	NT
141	54206/19	TISSUE LUNG	24.26	POSITIVE	NT
143	54208/19	TISSUE BONE MARROW	28.27	POSITIVE	NT
145	54208/19	TISSUE SPLEEN	27.01	POSITIVE	NT
147	54210/19	TISSUE LUNG	27.27	POSITIVE	NT
149	54212/19	TISSUE BONE MARROW	28.56	POSITIVE	NT
151	54212/19	TISSUE SPLEEN	24.33	POSITIVE	NT
153	54212/19	TISSUE KIDNEY	27.07	POSITIVE	NT
155	54212/19	TISSUE LUNG	23.47	POSITIVE	NEGATIVE
157	54214-5/19	TISSUE BONE MARROW	33.57	POSITIVE	NEGATIVE
159	56264-2/19	BLOOD SERUM	23.41	POSITIVE	NEGATIVE
160	56264-3/19	BLOOD SERUM	23.15	POSITIVE	NEGATIVE
161	56265-1/19	TISSUE BONE MARROW	23.22	POSITIVE	NEGATIVE
163	56265-2/19	TISSUE BONE MARROW	24.9	POSITIVE	NT
165	56265-3/19	TISSUE BONE MARROW	22.6	POSITIVE	NT
167	56941-2/19	TISSUE BONE MARROW	26.17	POSITIVE	NEGATIVE
169	59071-1/19	HOMOGENATE BONE MARROW	26.76	POSITIVE	NT
170	59071-2/19	HOMOGENATE BONE MARROW	23.3	POSITIVE	NT
171	59071-3/19	HOMOGENATE BONE MARROW	26.14	POSITIVE	NT
172	59071-5/19	HOMOGENATE BONE MARROW	23.52	POSITIVE	NT
173	59071-6/19	HOMOGENATE BONE MARROW	32.96	POSITIVE	NT
174	59071-7/19	HOMOGENATE BONE MARROW	20.61	POSITIVE	NT
175	59071-8/19	HOMOGENATE BONE MARROW	19.41	POSITIVE	NEGATIVE
176	59071-9/19	HOMOGENATE BONE MARROW	24.07	POSITIVE	NT
177	59071-11/19	HOMOGENATE BONE MARROW	27.02	POSITIVE	NT
178	60342-1/19	TISSUE BONE MARROW	28.56	POSITIVE	NEGATIVE

NT = not tested

- (a) **UPL-real time PCR** → Real time PCR test described by Fernández et al., 2013 based on the Universal Probe Library (UPL) and described in the OIE Manual of diagnosis for ASF (Chapter 3.8.1. OIE edition 2019).
- (b) **VI-HAD** → Virus isolation and haemadsorption test on PBM cells as is described in the OIE Manual of diagnosis for ASF (Chapter 3.8.1. OIE edition 2019).

2. **ASF antibody detection** → a total of **110 samples** were tested by IPT, **confirming the presence of antibodies in 21** that correspond to **14 out of the 84 wild boar tested (16.6%)**. The results obtained in ASF antibody detection by IPT are summarized in **table 3**.



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Table 3→ antibody detection by IPT					
Sample ID CISA	ID POLAND'S NRL		IPT ^a		Type of sample tested
	Id animal	Type of sample	Titer	Result	
1	12860-3/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
3	14751-1/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
5	14751-6/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
7	14988-1/18	BONE MARROW	1/5	WEAK ¹	HOMOGENATE
9	14988-2/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
11	14988-4/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
13	14988-5/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
15	14988-7/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
17	14988-8/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
19	15286-3/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
20	15390-1/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
22	15390-2/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
24	15390-3/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
26	15392/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
28	17567-2/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
30	17567-3/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
32	17561/8	SPLEEN	NEG	NEGATIVE	Exudate
34	38656/18	BONE MARROW	1/5	WEAK	HOMOGENATE
36	40176-1/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
37	40586-1/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
38	40663-1/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
40	41877/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
41	41879-2/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
43	50033/18	BONE MARROW	1/5	WEAK	HOMOGENATE
45	51617/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
46	53500-1/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
48	53500-2/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
50	52838-2/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
52	55233-1/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
54	55233-3/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
56	55233-4/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
58	56247/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
60	75885-1/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
62	75885-2/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
64	76038/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
66	76040/18	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
68	16235-1/18	SERUM	NEG	NEGATIVE	SERUM
69	24513-1/18	SERUM	NEG	NEGATIVE	SERUM
70	26020-2/18	SERUM	NEG	NEGATIVE	SERUM
71	26376-4/18	SERUM	NEG	NEGATIVE	SERUM
72	29838-11/18	SERUM	NEG	NEGATIVE	SERUM
73	75806-2/18	SERUM	NEG	NEGATIVE	SERUM
77	29267/19	LUNG	NEG	NEGATIVE	HOMOGENATE
78	32881/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE



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Sample ID CISA	ID POLAND'S NRL		IPT ^a		Type of sample tested
	Id animal	Type of sample	Titer	Result	
79	32881/19	SPLEEN	NEG	NEGATIVE	HOMOGENATE
80	32881/19	KIDNEY	NEG	NEGATIVE	HOMOGENATE
81	34250/19	KIDNEY	1/20	WEAK	HOMOGENATE
82	34251/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
83	35786/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
84	36159-1/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
85	36938-1	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
86	36938-2	BONE MARROW	1/80	POSITIVE	HOMOGENATE
87	36938-3	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
88	37465-2/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
89	37465-3/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
90	38346/19	BONE MARROW	1/20	WEAK	HOMOGENATE
92	51469/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
94	54186/19	LIVER	NEG	NEGATIVE	EXUDATE
96	54188/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
98	54188/19	SPLEEN	NEG	NEGATIVE	HOMOGENATE
100	54188/19	LUNG	1/5	WEAK	EXUDATE
102	54188/19	LIVER	NEG	NEGATIVE	HOMOGENATE
104	54190/19	SPLEEN	1/5	WEAK	EXUDATE
106	54190/19	LUNG	NEG	NEGATIVE	HOMOGENATE
108	54190/19	LIVER	NEG	NEGATIVE	HOMOGENATE
110	54192/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
112	54192/19	SPLEEN	NEG	NEGATIVE	HOMOGENATE
114	54192/19	LIVER	NEG	NEGATIVE	HOMOGENATE
116	54194/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
118	54194/19	SPLEEN	1/5	WEAK	EXUDATE
120	54194/19	LUNG	1/5	WEAK	EXUDATE
122	54194/19	LIVER	1/5	WEAK	EXUDATE
124	54198/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
126	54198/19	SPLEEN	NEG	NEGATIVE	EXUDATE
128	54198/19	LUNG	NEG	NEGATIVE	HOMOGENATE
130	54202/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
132	54202/19	SPLEEN	NEG	NEGATIVE	HOMOGENATE
133	54204/19	BONE MARROW	1/5	WEAK	HOMOGENATE
135	54204/19	SPLEEN	1/80	POSITIVE	EXUDATE
137	54204/19	KIDNEY	1/320	POSITIVE	EXUDATE
139	54204/19	LUNG	1/160	POSITIVE	EXUDATE
141	54206/19	LUNG	1/2560	POSITIVE	EXUDATE
143	54208/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
145	54208/19	SPLEEN	NEG	NEGATIVE	EXUDATE
147	54210/19	LUNG	1/2560	POSITIVE	EXUDATE
149	54212/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
151	54212/19	SPLEEN	1/160	POSITIVE	EXUDATE
153	54212/19	KIDNEY	1/160	POSITIVE	EXUDATE
155	54212/19	LUNG	1/20	WEAK	HOMOGENATE

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Sample ID CISA	ID POLAND'S NRL		IPT ^a		Type of sample tested
	Id animal	Type of sample	Titer	Result	
157	54214-5/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
159	56264-2/19	SERUM	NEG	NEGATIVE	SERUM
160	56264-3/19	SERUM	NEG	NEGATIVE	SERUM
161	56265-1/19	BONE MARROW	1/160	POSITIVE	EXUDATE
163	56265-2/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
165	56265-3/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
167	56941-2/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
169	59071-1/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
170	59071-2/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
171	59071-3/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
172	59071-5/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
173	59071-6/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
174	59071-7/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
175	59071-8/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
176	59071-9/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
177	59071-11/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE
178	60342-1/19	BONE MARROW	NEG	NEGATIVE	HOMOGENATE

WEAK= IPT TITER < 1/80

- a) **EUURL-IPT** → indirect immunoperoxidase technique on E70MS infected cells using the protocol standardized and validated by the European Union Reference Laboratory (URL), [PNT/CISA/PPA/IPT/1] and in the OIE Manual of diagnosis for ASF (Chapter 3.8.1. OIE edition 2019).

The eight sera were analyzed by the ELISA test. Only one gave a positive result but it was not confirmed by either IPT or IB (Table 4).

Sample ID CISA	ID POLAND'S NRL		ING-ELISA ^(a)		OIE-IB ^(b)	EUURL-IPT ^(c)
	Id animal	Type of sample	O.D value	Result		
68	16235-1/18	SERUM	0.12	POSITIVE	NEGATIVE	NEGATIVE
69	24513-1/18	SERUM	1.273	NEGATIVE	NT	NEGATIVE
70	26020-2/18	SERUM	1.186	NEGATIVE	NT	NEGATIVE
71	26376-4/18	SERUM	1.395	NEGATIVE	NT	NEGATIVE
72	29838-11/18	SERUM	1.156	NEGATIVE	NT	NEGATIVE
73	75806-2/18	SERUM	1.177	NEGATIVE	NT	NEGATIVE
159	56264-2/19	SERUM	0.848	NEGATIVE	NT	NEGATIVE
160	56264-3/19	SERUM	1.005	NEGATIVE	NT	NEGATIVE

WEAK= IPT Titre <1/80; NT = not tested.

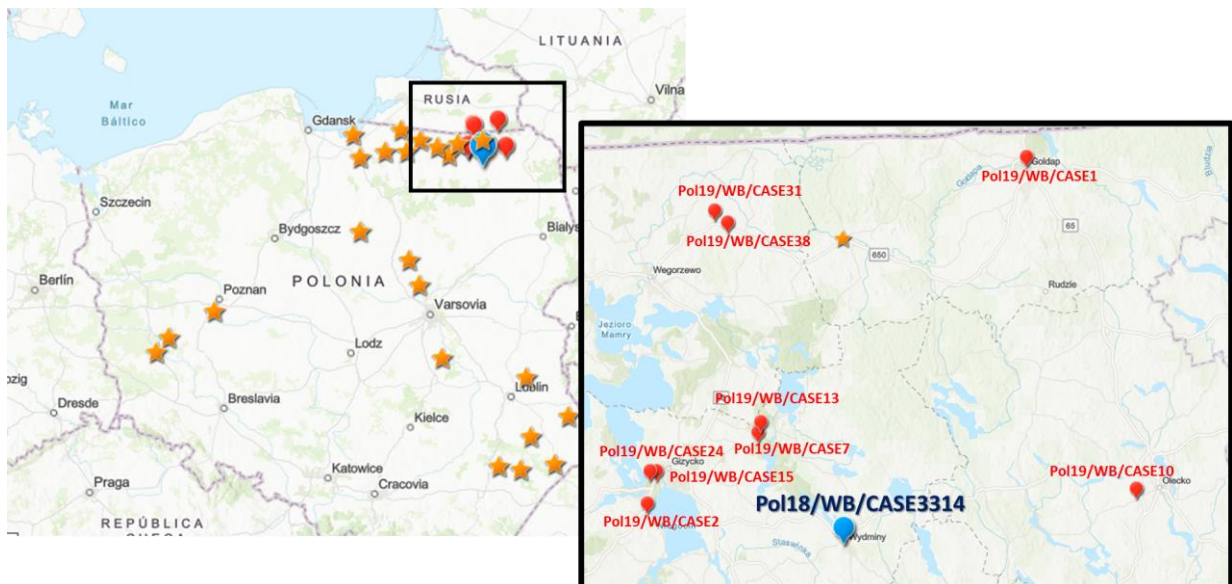
- (a) **ELISA INGENASA K3** → commercial ELISA kit Ingezim PPA Compac (11.PPA k3). [PNT/CISA/PPA/ELISA/2]
 (b) **OIE-IB** → Immunoblotting described in the OIE Manual of diagnosis for ASF (Chapter 3.8.1. OIE edition 2019) [PNT/CISA/PPA/IB/1]
 (c) **EUURL-IPT** → indirect immunoperoxidase technique on E70MS infected cells using the protocol standardized and validated by the European Union Reference Laboratory (URL), [PNT/CISA/PPA/IPT/1] and in the OIE Manual of diagnosis for ASF (Chapter 3.8.1. OIE edition 2019).

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3. **ASFV molecular characterization*** → Genetic characterization of ASFV was achieved in **41 PCR positive wild boar representatives of the 49 wild boar cases received**. The cases, 1836, 1979, 2100, 2136, 2155, 2198, 2576 and 3276 were not sequenced due to the negative result obtained with the PCR tests.

Sequence analysis of the CVR within the *B602L* gene and the intergenic region between the MGF 505 *9R* and the *10R* genes (MGF) placed the ASFVs within the **GII-CVR1** and **GII-MGF -1 variants**, respectively, mostly circulating in the EU countries. However, the amplification of the **intergenic region between the 173R and 1329L genes** (*IGRI73R-1329L*), generated amplicons ranging from around 367 to 387 bp from the Polish WB viruses. The sample ID 60 (*Pol18/WB CASE3314*) gave a PCR amplicons of 387bp. The nucleotide sequence analysis of this PCR product revealed the presence in this sample of the additional tandem repeat sequence insertion of 10 nts **TATATAGGAA** characteristic of the **GII-IGR_{173R-1329L}-4 variant** previously identified in other EU ASFV isolates collected in North Poland from cases occurred in January in 2019 (**figure 1**). The remaining viruses belonged to the *GII-IGR_{173R-1329L}-2* mostly circulating in the EU countries

Figure 1: map localization of the **Polish 2018 wild boar ASFV (case 3314)** identified in this study and the **Polish 2019 wild boar ASFVs** belonging to the *IGR_{173R-1329L}-4* variant. The *GII-IGR_{173R-1329L}-2* ASFVs are marked in yellow.





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Table 5 → Summary of the molecular characterization results from Eastern and Central European ASFV isolates 2007-2020					
COUNTRY	YEAR	P72 GENOTYPE	CVR SUBTYPING	IGR _{I73R-1329L} SUBTYPING	IGR _{MGF} SUBTYPING
Georgia	2007	II	CVR1	IGR-1	MGF -1
Armenia	2007	II	CVR1	IGR-1	MGF -1
Azerbaijan	2008	II	CVR1	IGR-1	MGF -1
Russia Federation	2007-2012	II	CVR1	IGR-1	MGF -1
	2012-2017	II	CVR1	IGR-1 + IGR-2	MGF -1 + MGF -2 + MGF -3
Ukraine	2012, 2015	II	CVR1	IGR-2	MGF -1
Belarus	2013	II	CVR1	IGR -2	MGF -1
Estonia	2014	II	CVR1	IGR-2	MGF -1
	2015-2018	II	CVR1 + CVR1SNP1* + CVR 2	IGR-2	MGF -1
Latvia	2014-2018	II	CVR1	IGR-2	MGF -1
	2017	II	CVR1	IGR-2	MGF -1+ MGF -1V** + MGF -2
	2018	II	CVR1	IGR-2	MGF -1+ MGF -1V** + MGF -2
	2019	II	CVR1	IGR-2	MGF -1+ MGF -2
Lithuania	2014-2016	II	CVR1	IGR-2	MGF -1
	2017-2019	II	CVR1+CVR1SNP3*	IGR-2	MGF -1+ MGF -4 + MGF -5
Poland	2014-2015	II	CVR1	IGR-2	MGF -1
	2016	II	CVR1	IGR-2	MGF -1 + MGF -2
	2017	II	CVR1 + CVR1SNP2*	IGR-1 + IGR-2+ IGR3	MGF -1 + MGF -2
	2018	II	CVR1	IGR-2+ IGR3+ IGR4	MGF -1
	2019	II	CVR1	IGR-2+ IGR4	MGF -1
Moldova	2016-2018	II	CVR1	IGR-2	MGF -1
Czech Republic	2017-2018	II	CVR1	IGR-2	MGF -1
Romania	2017-2018	II	CVR1	IGR-2	MGF -1
Hungary	2018-2019	II	CVR1	IGR-2	MGF -1
Bulgaria	2018-2020	II	CVR1	IGR-2	MGF -1
Belgium	2018	II	CVR1	IGR-2	MGF -1
Serbia	2019-2020	II	CVR1	IGR-2	MGF -1
Slovakia	2019	II	CVR1	IGR-2	MGF -1
Greece	2020	II	CVR1	IGR-2	MGF -1
Germany ¹	2020	II	CVR1	IGR-2	MGF -1

1: sequence done at the German's NRL (Genbank Acc Nº LR899193.1)

*single nucleotide polymorphism within the CVR 1 variant.

**multigene family 505 1-(MGF-1) variant.



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CONCLUSION*

1. The **presence of ASF was confirmed** by ASFV genome detection and/or antibody detection in **77 out of the 84 wild boar cases received** from Poland since **March 2018 up to the December 2019**. Samples were from the regions of Warmińsko-Mazurskie (northern Poland), Kujawsko-Pomorskie (mid-northern Poland), Lubuskie, (west-central Poland), Wielkopolskie (west-central Polands), Mazowieckie (central Poland), Lubelskie (eastern Poland) and Podkarpackie (South-eastern corner of Poland).
2. The **CVR subtyping** clustered the Polish wild boar viruses within **the CVR-1 variant** majority circulating in the Eastern European countries since the first introduction in Georgia in 2007.
3. The **intergenic region (IGR I73R-I329L)** subtyping placed the Polish wild boar viruses from case 3314 collected in Northern Poland on December 2018, within the variant **IGR I73R-I329L variant 4 (IGR-4)** previously identified at the EURL in the cases occurred in 2019 (cases 1, 2, 10, 13, 15, 24 31 and 38) also from the same region.
4. The remaining wild boar viruses were classified within **IGR I73R-I329L variant 2 (IGR-2)** identical to that responsible of the cases occurred in wild boar and in domestic pigs in the EU countries since the ASFV entrance into the EU in 2014 and initially discovered in certain regions of the Russian Federation in 2012 (from the data available at the EURL).
5. The **MGF 505 subtyping** placed the Polish wild boar viruses within the **MGF variant 1 (MFG-1)** identical to that present in most ASFVs isolated since 2014 from wild boar and domestic pigs in the EU countries, as well as in the ASFV isolates available at the EURL collected from the outbreaks occurred in Belarus in 2013, Ukraine 2012 and 2015 and Moldova in 2016, 2017, 2018 (available at the EURL).

The **table 6** summarizes the results obtained in each animal combining virus and antibody detection tests, as well as the genotyping results.



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Table 6→ASF final diagnostic result

DATE OF SAMPLING	CASE	ID WILD BOAR	VIRUS DETECTION	ANTIBODY DETECTION	ASFV ISOLATE	GENOTYPING			
						GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
12/03/2018	CASE1703	12860-3/18	POSITIVE	NEGATIVE	POL18/WB CASE1703	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
16/03/2018	CASE1774	14988-1/18	POSITIVE	WEAK	POL18/WB CASE1774	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
16/03/2018	CASE1774	14988-2/18	POSITIVE	NEGATIVE	Not sequenced				
16/03/2018	CASE1774	14988-4/18	POSITIVE	NEGATIVE	Not sequenced				
16/03/2018	CASE1774	14988-5/18	POSITIVE	NEGATIVE	Not sequenced				
16/03/2018	CASE1774	14988-7/18	POSITIVE	NEGATIVE	Not sequenced				
16/03/2018	CASE1774	14988-8/18	POSITIVE	NEGATIVE	Not sequenced				
19/03/2018	CASE1746	14751-1/18	POSITIVE	NEGATIVE	POL18/WB CASE1746	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
19/03/2018	CASE1746	14751-6/18	POSITIVE	NEGATIVE	Not sequenced				
21/03/2018	CASE1795	15392/18	POSITIVE	NEGATIVE	POL18/WB CASE1795	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
21/03/2018	CASE1793	15390-1/18	POSITIVE	NEGATIVE	POL18/WB CASE1793	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
21/03/2018	CASE1794	15390-2/18	POSITIVE	NEGATIVE	POL18/WB CASE1794	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
21/03/2018	CASE1775	15286-3/18	POSITIVE	NEGATIVE	POL18/WB CASE1775	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
21/03/2018	CASE1794	15390-3/18	POSITIVE	NEGATIVE	Not sequenced				
03/04/2018	CASE1865	17567-3/18	POSITIVE	NEGATIVE	POL18/WB CASE1865	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
03/04/2018	CASE1865	17567-2/18	POSITIVE	NEGATIVE	Not sequenced				
03/04/2018	CASE1865	17561/18	POSITIVE	NEGATIVE	Not sequenced				
07/05/2018	CASE1836	16235-1/18	NEGATIVE	NEGATIVE	Not sequenced				
10/05/2018	CASE2100	24513-1/18	NEGATIVE	NEGATIVE	Not sequenced				
14/05/2018	CASE2136	26020-2/18	NEGATIVE	NEGATIVE	Not sequenced				
23/05/2018	CASE2155	26376-4/18	NEGATIVE	NEGATIVE	Not sequenced				
03/07/2018	CASE2458	38656/18	POSITIVE	WEAK	POL18/WB CASE2458	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
12/07/2018	CASE2548	40663-1/18	POSITIVE	NEGATIVE	POL18/WB CASE2548	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
12/07/2018	CASE2543	40586-1/18	POSITIVE	NEGATIVE	POL18/WB CASE2543	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
14/07/2018	CASE2574	41877/18	POSITIVE	NEGATIVE	POL18/WB CASE2574	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
14/07/2018	CASE2576	41879-2/18	NEGATIVE	NEGATIVE	Not sequenced				
15/07/2018	CASE2542	40176-1/18	POSITIVE	NEGATIVE	POL18/WB CASE2542	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
23/08/2018	CASE2766	50033/18	POSITIVE	WEAK	POL18/WB CASE2766	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
03/09/2018	CASE2820	52838-2/18	POSITIVE	NEGATIVE	POL18/WB CASE2820	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
06/09/2018	CASE2778	51617/18	POSITIVE	NEGATIVE	POL18/WB CASE2778	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
06/09/2018	CASE2819	53500-2/18	POSITIVE	NEGATIVE	POL18/WB CASE2819	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
06/09/2018	CASE2819	53500-1/18	POSITIVE	NEGATIVE	Not sequenced				
13/09/2018	CASE2839	55233-3/18	POSITIVE	NEGATIVE	POL18/WB CASE2839	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
13/09/2018	CASE2850	56247/18	POSITIVE	NEGATIVE	POL18/WB CASE2850	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
13/09/2018	CASE2839	55233-1/18	POSITIVE	NEGATIVE	Not sequenced				
13/09/2018	CASE2839	55233-4/18	POSITIVE	NEGATIVE	Not sequenced				
13/12/2018	CASE3314	75885-1/18	POSITIVE	NEGATIVE	POL18/WB CASE3314	GII	CVR- 1	IGR _{173R-1329L} -4	MGF -1
13/12/2018	CASE3314	75885-2/18	POSITIVE	NEGATIVE	Not sequenced				
17/12/2018	CASE3310	76040/18	POSITIVE	NEGATIVE	POL18/WB CASE3310	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
17/12/2018	CASE3315	76038/18	POSITIVE	NEGATIVE	POL18/WB CASE3315	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
18/12/2018	CASE2198	29838-11/18	NEGATIVE	NEGATIVE	Not sequenced				
18/06/2019	CASE1298	29267/19	POSITIVE	NEGATIVE	POL19/WB CASE1298	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
03/07/2019	CASE1383	32881/19	POSITIVE	NEGATIVE	POL19/WB CASE1383	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
18/07/2019	CASE1436	34250/19	POSITIVE	WEAK	Not sequenced				
20/07/2019	CASE1436	34251/19	POSITIVE	NEGATIVE	POL19/WB CASE1436	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
29/07/2019	CASE1506	35786/19	POSITIVE	NEGATIVE	POL19/WB CASE1506	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
02/08/2019	CASE1526	36938-1	POSITIVE	NEGATIVE	Not sequenced				MGF1
02/08/2019	CASE1526	36938-2	POSITIVE	POSITIVE	Not sequenced				



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DATE OF SAMPLING	CASE	ID WILD BOAR	VIRUS DETECTION	ANTIBODY DETECTION	ASFV ISOLATE	GENOTYPING			
02/08/2019	CASE1502	36159-1/19	POSITIVE	NEGATIVE	POL19/WB CASE1502	GII	CVR- 1	IGR _{173R-1329L} -2	
07/08/2019	CASE1541	37465-2/19	POSITIVE	NEGATIVE	Not sequenced				
07/08/2019	CASE1526	36938-3	POSITIVE	NEGATIVE	POL19/WB CASE1526	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
13/08/2019	CASE1589	38346/19	POSITIVE	WEAK	POL19/WB CASE1589	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
13/08/2019	CASE1541	37465-3/19	POSITIVE	NEGATIVE	POL19/WB CASE1541	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
22/09/2019	CASE1912	51469/19	POSITIVE	NEGATIVE	POL19/WB CASE1912	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
16/11/2019	CASE1973	54186/19	POSITIVE	NEGATIVE	Not sequenced				
16/11/2019	CASE1973	54188/19	POSITIVE	WEAK	Not sequenced				
16/11/2019	CASE1973	54190/19	POSITIVE	WEAK	Not sequenced				
16/11/2019	CASE1973	54194/19	POSITIVE	WEAK	Not sequenced				
16/11/2019	CASE1979	54214-5/19	POSITIVE	NEGATIVE	Not sequenced				
16/11/2019	CASE1973	54192/19	POSITIVE	NEGATIVE	POL19/WB CASE1973	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
17/11/2019	CASE1977	54202/19	POSITIVE	NEGATIVE	Not sequenced				
17/11/2019	CASE1977	54206/19	POSITIVE	POSITIVE	Not sequenced				
17/11/2019	CASE1977	54208/19	POSITIVE	NEGATIVE	Not sequenced				
17/11/2019	CASE1978	54210/19	POSITIVE	POSITIVE	Not sequenced				
17/11/2019	CASE1978	54212/19	POSITIVE	POSITIVE	POL19/WB CASE1978	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
17/11/2019	CASE1976	54198/19	POSITIVE	NEGATIVE	POL19/WB CASE1976	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
17/11/2019	CASE1977	54204/19	POSITIVE	POSITIVE	POL19/WB CASE1977	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
25/11/2019	CASE2106	56265-2/19	POSITIVE	NEGATIVE	Not sequenced				
25/11/2019	CASE2106	56265-3/19	POSITIVE	NEGATIVE	Not sequenced				
25/11/2019	CASE2106	56265-1/19	POSITIVE	POSITIVE	POL19/WB CASE2106	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
25/11/2019	CASE2108	56264-2/19	POSITIVE	NEGATIVE	POL19/WB CASE2108	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
25/11/2019	CASE2109	56264-3/19	POSITIVE	NEGATIVE	POL19/WB CASE2109	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
26/11/2019	CASE2137	56941-2/19	POSITIVE	NEGATIVE	POL19/WB CASE2137	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
11/12/2019	CASE2219	59071-11/19	POSITIVE	NEGATIVE	Not sequenced				
11/12/2019	CASE2309	60342-1/19	POSITIVE	NEGATIVE	POL19/WB CASE2309	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1
18/06/2019	CASE3276	75806-2/18	NEGATIVE	NEGATIVE	Not sequenced				
NI	CASE2219	59071-1/19	POSITIVE	NEGATIVE	Not sequenced				
NI	CASE2219	59071-2/19	POSITIVE	NEGATIVE	Not sequenced				
NI	CASE2219	59071-3/19	POSITIVE	NEGATIVE	Not sequenced				
NI	CASE2219	59071-5/19	POSITIVE	NEGATIVE	Not sequenced				
NI	CASE2219	59071-6/19	POSITIVE	NEGATIVE	Not sequenced				
NI	CASE2219	59071-7/19	POSITIVE	NEGATIVE	Not sequenced				
NI	CASE2219	59071-9/19	POSITIVE	NEGATIVE	Not sequenced				
NI	CASE2219	59071-8/19	POSITIVE	NEGATIVE	POL19/WB CASE2219	GII	CVR- 1	IGR _{173R-1329L} -2	MGF1

(1) WEAK = PCR-UPL weak (Ct≥35). (2) WEAK = IPT weak titer < 1/80. NI= not information provided

REFERENCES

- Carmina Gallardo, Jovita Fernández-Pinero, Virginia Pelayo, Ismail Gazaev, Iwona Markowska-Daniel, Gediminas Pridotkas, Raquel Nieto, Paloma Fernández-Pacheco, Svetlana Bokhan, OlegNevolko, ZhannaDrozhzhe, Covadonga Pérez, Alejandro Soler, Denis Kolvasov, and Marisa Arias (2014). Genetic Variation among African Swine Fever Genotype II Viruses, Eastern and CeNTral Europe. *Emerg. Infect. Dis.* Volume 20, Number 9—September 2014.
- Elsukova, Aleksandra; Shevchenko, Ivan; Varentsova, Alisa; Zinyakov, Nikolay; Igolkin, Aleksey; Vlasova, Natalia. African swine fever (ASF), intergenic region, 9R/10R, NGS, tandem repeat sequences in the intergenic region MGF 505 9R/10R is a new marker of the genetic variability among ASF Genotype II virusessm, EPIZONE, 10th Annual Meeting, 27-29 September 2016, MADRID, SPAIN
- Fernández-Pinero J, Gallardo C, Elizalde M, Robles A, Gómez C, Bishop R, Health L, Couacy-Hymann E, Fasina FO, Pelayo V, Soler A, Arias M. Molecular diagnosis of African swine fever by a new real-time PCR using universal probe library (2013). *Transbound Emerg Dis* 60 (2013) 48–58. doi: 10.1111/j.1865-1682.2012.01317.x. Epub 2012 Mar 7.
- Gallardo C, Anchuelo R, Pelayo V, Poudevigne F, Leon T, Nzoussi J, Bishop R, Pérez C, Soler A, Nieto R, Martín H, Arias M. (2011). African swine fever virus p72 genotype IX in domestic pigs, Congo. *Emerg. Infect. Dis.* Aug; 17(8):1556-8.
- Goller KV, Malogolovkin AS, Katorkin S, Kolbasov D, Titov I, Höpfer D, et al. Tandem repeat insertion in African swine fever virus, Russia, 2012 [letter]. *Emerg Infect Dis.* 2015 Apr [date cited]. <http://dx.doi.org/10.3201/eid2104.141792>
- OIE. May 2019, posting date. Chapter 3.8.1. African swine fever (Infection with African swine fever virus). In *Manual of diagnostic tests and vaccines for terrestrial animals* 2012. World Organization for Animal Health, Paris, France https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.08.01_ASF.pdf



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In Valdeolmos, Madrid (Spain) 16th March 2021

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