**Table 1:** Identification of the ASFV isolates and design of the animal experiment.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **ASFV isolates origin** | | | | **Design of the animal experiment** | | | |
| **ID** | **HOST** | **Location** | **Collection date** | **Status** | **ID animal** | **Day of death (‡) or slaughtered (†)** | |
| Es16/WB/Viru8  (HAD) | Wild boar | Lääne-Viru county, Estonia | 30/11/2016 | Inoculated | **E1** | † | 10 dpi |
| **E5** | ‡ | 10 dpi |
| In contact | E2 | † | 76 dpe |
| E3 | ‡ | 15 dpe |
| E4 | † | 20 dpe |
| E6 | † | 76 dpe |
| Pol16/DP/OUT21 (HAD) | Domestic pig | Leśna, Lubań county Poland | 25/09/2016 | Inoculated | **P7** | ‡ | 8 dpi |
| **P12** | ‡ | 8 dpi |
| In contact | P8 | † | 14 dpe |
| P9 | † | 20 dpe |
| P10 | † | 13 dpe |
| P11 | † | 13 dpe |
| Lv17/WB/Rie1\* (non-HAD) | Wild boar | Engures, Smardesu parish, Latvia. | 01/02/2017 | Inoculated | **PW13** | † | 126 dpi |
|  | **PW17** | † | 45 dpi |
|  | In contact | PW14 | † | 126 dpe |
|  | PW15 | † | 101 dpe |
|  | PW16 | † | 25 dpe |
|  | PW18 | † | 101 dpe |

Dpi (days post infection); dpe (days post exposure)

\*Experiment previously described by Gallardo et al 2019a.

**Table 2:** Comparative results of the onset of the disease indicating the incubation period and survival rate in the domestic pigs infected with the HAD-Polish (Pol16/DP/OUT21), HAD-Estonia (Est16/WB/Viru8) or the non-HAD Latvian (Lv17/WB/Rie1) ASFVs.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ASFV isolate** | **Incubation period\* (±SD)** | | **SURVIVAL [Mean time to death days (±SD)]** | |
| **INOCULATED** | **CONTACT** | **INOCULATED** | **CONTACT** |
| Pol16/DP/OUT21 | 4.5±0.7 | 9±1 | 0% [8±0] | 0% [16.5± 2,4] |
| Es16/WB/Viru8 | 5.5±0.7 | 13± 0 | 0% [10±0] | 50% [18± 1,4] |
| Lv17/WB/Rie1 | 8± 0 | 11,5± 0 | 100% | 100% |

\*days from infection to onset of clinical signs; SD = standard deviation

**Table 3:** Comparative results of the onset of ASFV DNA (PCR) detection and virus isolation (VI) in the inoculated (A) and in the in contact (B) domestic pigs infected with the HAD-Polish (Pol16/DP/OUT21), HAD-Estonia (Est16/WB/Viru8) or the non-HAD Latvian (Lv17/WB/Rie1) ASFVs. Number in brackets indicates the percentages of positive animals at the end of the observation period.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 1. **Inoculated pigs** | | | | | | |
| **ASFV isolate** | **ASFV genome detection**1 | | | **ASFV virus isolation2** | | |
| **Blood** | **OPS** | **FCS** | **Blood** | **OPS** | **FCS** |
| Pol16/DP-OUT21 (Vir.) | 3±0 (100%) | 5±1.6 (100%) | 5±1.6 (100%) | 3±0 (100%) | 7±0 (100%) | 7±0 100%) |
| Es16/WB-Viru8 (Mod. Vir) | 3±0 (100%) | 7±0 (100%) | 7±0 (100%) | 7±0 (100%) | 7±0 (100%) | 7±0 (50%) |
| Lv17/WB/Rie1 (Att.) | 5±1.6 (100%) | 5±1.6 (100%) | Sporadic (100%) | 5±1.6 (100%) | Neg. | Neg. |
|  |  |  |  |  |  |  |
| 1. **In contact pigs** | | | | | | |
| Pol16/DP-OUT21 (Vir.) | 10±2.1 (100%) | 7±0 (100%) | 8.5±1.3 (100%) | 10±2.1 (100%) | 13±2.1 (100%) | 13±0  (25%) |
| Es16/WB-Viru8 (Mod. Vir) | 10±2.1 (100%) | 8.5±1.2 (100%) | 14.5±1.2 (75%) | 13±0 (100%) | 10±0 (75%) | Sporadic (25%) |
| Lv17/WB/Rie1 (Att.) | 18±2.7 (75%) | 14.5±4.7 (100%) | Sporadic (75%) | 17.6±4 (50%) | Sporadic (25%) | Neg. |

(1) ASFV genome detection indicating first positive finding in days ±standard deviation (SD) versus (% positive animals); (2) ASFV virus isolation detection indicating first positive isolation in days ±standard deviation (SD) versus (% positive animals)

**Table 4.** Percentage of PCR-positive samples and virus isolation (VI) regarding to ASFV detection in blood, oropharyngeal swabs (OPS), and fecal swabs (FCS) in the three groups of infected animals with the virulent ***(Vir.)***, moderately virulent ***(Mod. Vir.)*** or attenuated ***(Att.)*** ASFVs at different days-period.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group/ASFV** | **Test** | **1-20 days** | | | **>21 days** | | | **TOTAL** | | |
| **BLOOD** | **OPS** | **FCS** | **BLOOD** | **OPS** | **FCS** | **BLOOD** | **OPS** | **FCS** |
| **Vir.** Pol16/DP/OUT21 | **PCR** | 68.2% | 77.3% | 54.5% |  |  |  | 68.2% | 77.3% | 54.5% |
| **VI** | 86.7% | 53% | 25% |  |  |  | 86.7% | 53% | 25% |
| **Mod. Vir.** Est16/WB/Viru8 | **PCR** | 61.5% | 69.2% | 26.9% | 97% | 30% | 0% | 80.4% | 48.2% | 12.5% |
| **VI** | 81% | 67% | 57% | 31% | 0% | 0% | 48.9% | 44.4% | 57.1% |
| **Att.** Lv17/WB/Rie1 | **PCR** | 37.7% | 50% | 10% | 29% | 32% | 8% | 31.1% | 36.9% | 8.7% |
| **VI** | 100% | 7% | 0% | 10% | 0% | 0% | 40.6% | 2.6% | 0% |

**Table 5.** African swine fever virus (ASFV) comparative antibody detection in serum samples by ELISA and IPT indicated the first positive finding in days ±standard deviation (SD) and the percentage of positives domestic pigs

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ASFV isolate** | **Inoculated pigs (IP)** | | **In contact pigs (CP)** | |
| **ELISA** | **IPT** | **ELISA** | **IPT** |
| Pol16/DP/OUT21 | NEG. | 7±0 (100%) | 16±0 (25%) | 11.5 ±1.2 (75%) |
| Es16/WB/Viru8 | NEG. | 7±0 (50%) | 16±0 (50%) | 16±0 (75%) |
| Lv17/WB/Rie1 | 10 (100%) | 7±0 (100%) | 19.5±3.6 (100%) | 19.5±3.6 (100%) |