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Survival at +4°C, +20°C and +37°C of Italian genotype I and II African Swine Fever strains

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INTRODUCTION

- African swine fever (ASF) is a viral disease affecting domestic and wild pigs, causing a haemorrhagic fever-like disease with exceptionally high lethality;
- ASF virus (ASFV) is a large, enveloped dsDNA belonging to the *Asfarviridae* family, Asfivirus genus (**Fig. 1**);
- ASF is a major threat to animal health with high economic direct and indirect impacts to the swine industry worldwide;
- ASF is transmitted through direct and indirect contacts with infected animals, ingestion of animal products, and contact with contaminated fomites;
- The environmental contamination during an outbreak in domestic and wild pigs is a critical issue in limiting the disease spread;
- ASF persistence and spread is therefore a function of the virus resistance to physical and chemical factors;
- In the last decade, the global ASFV spread and the absence of vaccines have directed research towards the field of vaccinology and studies on virus resistance has remained as a marginal research topic.

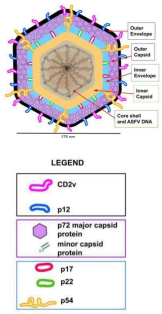

Figure 1. Schematic illustration of ASFV virion.

MATERIALS & METHODS

Three ASFV strains were selected:
1. BA71/V, genotype I, the laboratory-adapted (LA), as a reference strain;
2. Sardegna49, genotype I, isolated in Sardinia in 2008;
3. Genova22, genotype II, isolated in wild boar in Italy in 2022.

- Viruses were exposed at +4°C, +20°C, and +37°C and tested at day 0, 7 and 15.
- For each exposure time, three independent virus aliquots were prepared and subsequently tested for residual infectivity by virus titration on cell cultures according to the Spaerman-Karber formula.
- A two-way ANOVA test was run to study the effect of temperature and time-points on viral titres. Post hoc analysis was carried out, and significance levels were adjusted using the Bonferroni correction.

AIM OF THE STUDY

The present study aimed at generating data on survival of currently circulating ASFV strains of genotype I and II detected in Italy at three different temperatures for fifteen days.

RESULTS

A statistically significant difference in TCID50 was identified between the temperatures (p-value<0.01) and between time points (p-value<0.0001) (**Fig. 2**).
In particular, the TCID50 at +37°C was found to be on average lower than at +4 and 20°C. The average TCID50 at 15 days was lower than the average at 0 and 7 days; the average at 7 days was lower than the average at 0 days.

A statistically significant difference in HAD50 was identified between the temperatures (p-value<0.001) and between the times (p-value<0.001) (**Fig. 3**). In particular, the HAD50 at +37°C was found to be on average lower than at +4 and +20°C. The average HAD50 at 15 days was lower than the average at 0 and 7 days. From the assessment of contrasts at +4 and +20°C, no significant differences over time were observed. At +37°C, a statistically significant reduction was noted at 15 days.

A statistically significant difference in HAD50 was identified between the temperatures (p-value<0.0001) and between the times (p-value<0.0001) (**Fig. 4**). In particular, HAD50 at +37°C was found to be lower on average compared to +4 and +20°C for all tested times. At +37°C, the decay of viral titers occurs more rapidly between 7 and 15 days compared to 0 and 7 days, and this decrease is statistically significant.

BA71V, Genova22 (II), Sardegna49 (I)

Days	Virus		
	Ba71V	Genova22	Sardegna49
0	5.67	5.2	4.87
7	4.75	4.7	3.28
15	4.25	3.37	0

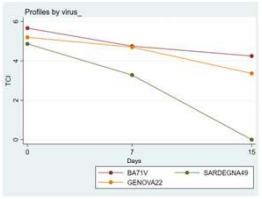

Figure 5. The analyzed data refer to the base 10 logarithms of virus titrations for the viruses BA71V, Genova22 and Sardegna49 exposed to 37°C to assess the possible mean differences in titers between the viruses and the days.

From the assessment of contrasts, for the virus BA71V, a significant difference emerged between 0 and 7 days, maintained until 15 days, but not between 7 and 15 days. For the Sardegna49 strain, the reduction was statistically significant both between 0 and 7 days and between 7 and 15 days. For the virus Genova22, significant differences were observed only at 15 days compared to 0 and 7 days (**Fig. 5**).

BA71/V (LA)

Days	Temperature (°C)		
	4	20	37
0	5.67	5.92	5.67
7	5	5.17	4.75
15	5	4.67	4.25

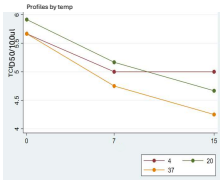

Figure 2. (*above*) TCID50 averages for time and temperature; (*below*) TCID50 trend for temperatures (4, 20, 37°C) over time for BA71/V.

Genova22 (II)

Days	Temperature (°C)		
	4	20	37
0	5.03	5.28	5.2
7	5.2	4.78	4.7
15	4.95	4.87	3.37

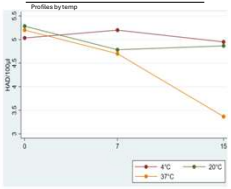

Figure 3. (*above*) HAD50 averages for time and temperature; (*below*) HAD50 trend for temperatures (4, 20, 37°C) over time for Genova22.

Sardegna49 (I)

Days	Temperature (°C)		
	4	20	37
0	4.87	4.95	4.87
7	4.95	4.78	3.28
15	4.97	4.78	0

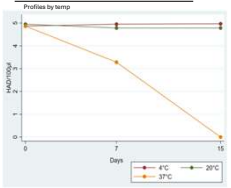

Figure 4. (*above*) HAD50 averages for time and temperature; (*below*) HAD50 trend for temperatures (4, 20, 37°C) over time for Sardegna49.

CONCLUSIONS

- All ASFV strains tested remain infectious following 15 days post exposure at +4°C and +20°C.
- At +37°C following 15 days the genotype I ASFV Sardegna49 was completely inactivated, while the other tested ASFV remained infectious. The decrease in virus titres at +37°C was statistically significantly dependent on the strain and the time; differences were detected at each time points for genotype I, between 7 and 15 days for the genotype II and between 0 and 7 days for the laboratory adapted ASFV.
- Comparing survival curves of each ASFV, a statistically significant difference was observed among temperatures and time points, indicating a higher survival rates at +4°C and +20°C than +37°C.
- This study partially confirms literature data on ASFV prolonged survival at physical factors such as temperature, suggesting a potential difference between Italian genotype I and II and the higher resistance of the currently circulating genotype II that must be further investigated testing additional ASFV strains. Such studies are pivotal to understand the role of survival characteristics on ASFV transmission.

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