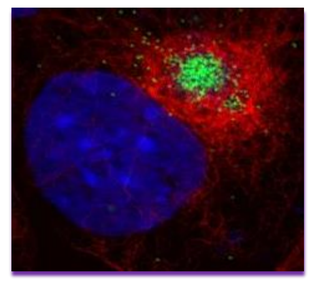

Comparative study of protection in pigs immunised by different routes and doses with the naturally attenuated African swine fever virus isolate OUR T88/3 and role of immunomodulatory cytokines.

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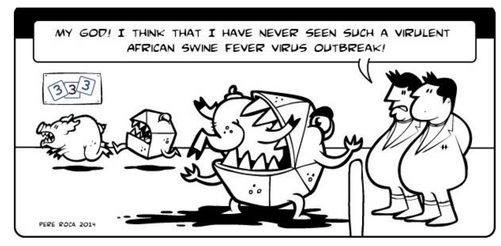
Introduction

- African swine fever (ASF) is a devastating disease of domestic swine, with a mortality rate reaching 100% for highly pathogenic isolates.
- The apparently unstoppable spread of ASF throughout the trans-Caucasus region and Russia since its introduction in 2007, as well as the recent incursions into Eastern Europe has highlighted the need for an effective vaccine to help control the disease.
- Control relies on rapid diagnosis, implementation of quarantine and slaughter of pigs.
- Despite the safety concerns raised by the use of a live attenuated vaccine (reversion to a virulent form or capability of causing subclinical or chronic ASF forms), to date, these vaccines have been the only form capable of conferring a reliable and effective protection against infections with homologous and occasionally heterologous virulent isolates of ASFV.



ABSTRACT

Comparative assessments were utilised to demonstrate differences between various combinations of doses (10^3 ,10^4 and 10^5 TCID50/ml), and routes of immunisation (intramuscular and intranasal) of low virulent African swine fever virus (ASFV) OURT88/3 in pigs.

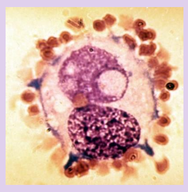
Intranasal immunisations with low and moderate doses (10^3 and 10^4 TCID50/ml), provided complete protection (100%), mild and transient clinical signs and transient, moderate virus genome levels in blood against challenge with virulent genotype I OURT88/1 isolate.

In contrast, pigs immunised intramuscularly with low and moderate doses (10^3 and 10^4 TCID50/ml) displayed lower protection rates (50 to 66%). Nevertheless, protected animals showed no clinical signs, and only low or undetectable levels of virus genome in blood throughout the study.

Materials and Methods

- *Cells and viruses:*

Viruses were grown and titrated in primary macrophage cultures. Titres are presented as (TCID50/ml).



- *Experimental design:*

Animal experiments were conducted in BSL-3 facilities at CReSA (Barcelona, Spain), according to regulated procedures from the Animals (Scientific Procedures) Act 1986

Experimental group	No. of pigs	Immunisation route	Immunisation dose of OUR T88/3 ($TCID_{50}$/ml)	Challenge by IM inoculation of OUR T88/1 ($TCID_{50}$/ml)
A	n=6	IM	10^3	10^4
B	n=6	IN	10^3	10^4
C	n=6	IM	10^4	10^4
D	n=6	IN	10^4	10^4
E	n=6	IM	10^5	10^4
F	n=6	IN	10^5	10^4
G (Control)	n=3	-	-	10^4

IN: Intranasal immunisation; IM: intramuscular immunisation

- *Sampling, clinical and post-mortem examination:*

Rectal temperatures and clinical signs were monitored daily. EDTA blood and serum samples were collected on day (0, 3, 5, 7, 14 and 21) post-immunisation and day (3, 5, 7, 14 and 19) post-challenge.

Non-protected pigs were euthanized at a specified endpoint, while protected pigs were euthanized at 19 dpc.

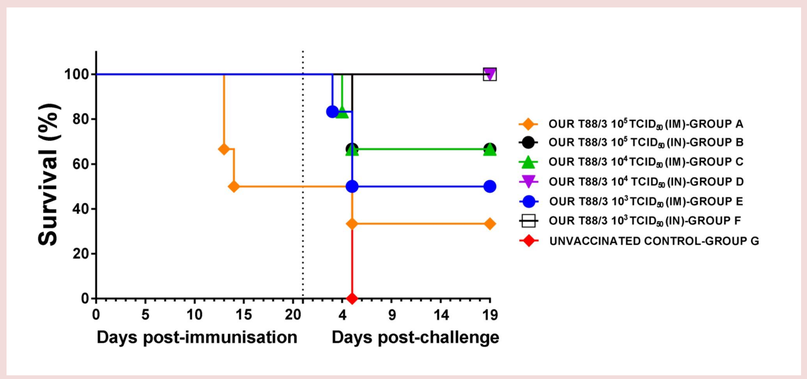
- *ASFV detection and immune response evaluation:*

Blood and tissues samples were analysed for ASFV genome detection by quantitative PCR (qPCR) (King et al., 2003).

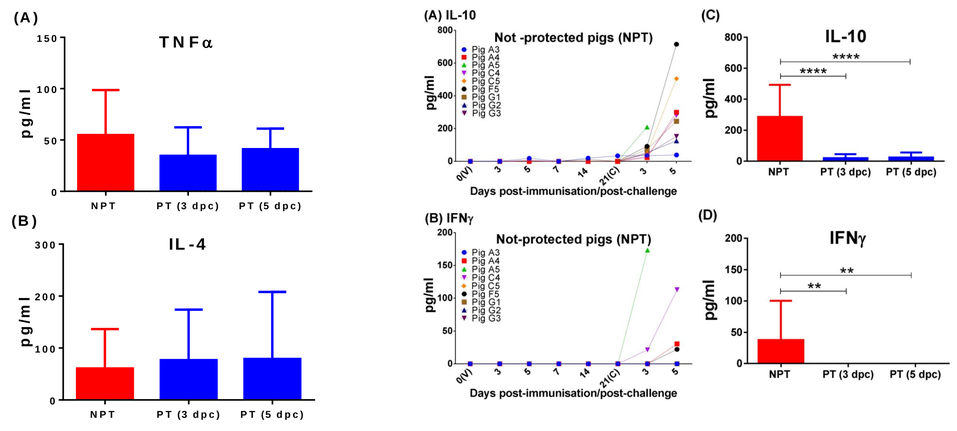
Serum samples were assayed by commercial ELISA kits (Blocking ELISA), for detection of ASFV-specific antibodies against VP72 (INGEZIM PPA Compac, Ingenasa), or porcine immunoregulatory cytokines (TNFα, IFNγ, IL-4 and IL-10) (R&D Systems).

Results

- Intranasal immunisations provided 100% protection in pigs vaccinated with low and moderate doses (10^3 and 10^4 $TCID_{50}$), and 66% protection in pigs vaccinated with high dose (10^5) of OURT88/3.
- Intramuscular immunisations induced lower protection with a survival rate of 50% and 66 % in pigs vaccinated with OURT88/3 (10^3 $TCID_{50}$) and (10^4 $TCID_{50}$) respectively



- Surviving animals (viraemic), had low virus titre on termination and no virus was detected in the tissues.
- Immunised animals that died, had high virus titres (10^7 or 10^8)
- Protected pigs did not display changes in serum cytokine levels, whereas an increase of IL-10 and IFNγ appeared post challenge in non-protected pigs with acute ASF.



Conclusions

- Intranasal immunisations with 10^3 and 10^4 OURT88/3 provided 100% survival rate in vaccinated animals.
- Survival of pigs after challenge was associated with a balance between pro- (TNFα and IL-1β) and anti-inflammatory cytokines (IL-10), without participation of IFNγ.
- In contrast, animals that died showing an acute form of ASF displayed an imbalance linked to an exacerbated increase of IL-10 along with an anomalous antibody response.

Future work

- Further testing of vaccine candidates.
- Testing duration of immunity.
- Testing onset of immunity.

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