Vaccine strain ASFV-G-ΔI177L reverts quickly to virulence and negatively impacts reproductive performance of sows

Erwin van den Born[1], Ferenc Olasz[2,3], István Mészáros[2], Eszter Göltl[2,3], Barbara Oláh[2], Jui Joshi[1], Emma van Kilsdonk[1], Zoltán Zádori[2,3]

Introduction

- ASFV-G-ΔI177L (ΔI177L) is a live-attenuated recombinant ASFV strain and has the I177L gene partially deleted (1).
- ΔI177L protects vaccinated pigs against ASFV infection (2).
- Previously, ΔI177L was shown to be safe and remained phenotypically attenuated during 5 passages in pigs (3).
- The use of ΔI177L in a commercial vaccine in Vietnam in 2022 was associated with serious animal health problems, including mortality. Local health officials concluded that this was caused by non-compliance with vaccination guidelines (4).
- Since ΔI177L showed great promise as a vaccine strain, we set out to investigate its safety for pregnant sows and confirm its genetic and phenotypic stability during *in vivo* passaging (5).
- We also present the efficacy and safety profile of a new vaccine strain that we recently developed.

Safety of ΔI177L in pregnant sows

- Two multiparous sows were vaccinated at around 100 days in gestation (i.e. approx. 15 days before parturition) with a low dose of ASFV-G-ΔI177L (Table 1).
- Both vaccinated sows became viremic.
- One of the vaccinated sows and two unvaccinated control sows did not develop fever and ASF-related clinical signs.
- One of the vaccinated sows had elevated temperatures, was inactive, and did not drink and eat on day 7 and 8 dpv. By day 11 she was still eating less and moving slowly, but by day 17 she was fully recovered.
- Reproductive performance of the vaccinated sows was significantly affected (Table 1). Piglets of sow 2 did not survive beyond 9 days post farrowing (dpf), while only 4 piglets of sow 1 stayed alive until study end (22 dpf).
- All piglets born to the vaccinated sows showed ASF-related clinical signs and elevated temperatures (Fig. 1) and were viremic at birth (Fig. 2) possibly due to vertical transmission.

Table 1. Animal study 1 - Specifics of the pregnant sow study

Group	No. of sows	Inoculum	Inoculation volume, route	Inoculation dose	Survival rate sows	Survival rate born piglets
I	2	ASFV-G-ΔI177L	2 ml, IM, neck	$1.5 \cdot 10^2$ $TCID_{50}$	100% (2/2)	10% (4/40)
II	2	PBS	2 ml, IM, neck	-	100% (2/2)	83% (38/46)

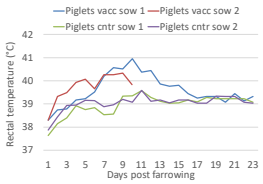

Figure 1. Average daily rectal temperature of piglets.

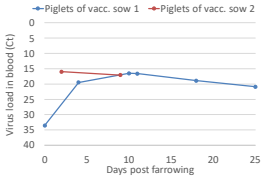

Figure 2. Virus load as determined by qPCR in blood taken from piglets born to the vaccinated sows.

Reversion to virulence of ΔI177L

- The *in vivo* passaging experiment was done according to PhEur 5.2.6 and VICH guideline 41 (Fig. 3). Male pigs were 7-9 weeks of age.
- Each of the two pigs in passage 1 received $1.5 \cdot 10^2$ $TCID_{50}$ of ΔI177L in 1 ml in the neck muscle.
- Animals in the next passage received 1 ml of undiluted blood from the animal(s) with the highest level of viremia.

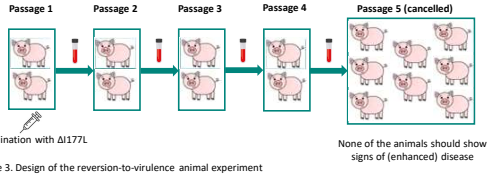

Figure 3. Design of the reversion-to-virulence animal experiment

- The data showed that whilst passage 1 (P1) was eventless, P2 pigs displayed some signs of disease and P3 and P4 pigs showed severe ASF clinical signs and high fever, concomitant with an strong increase in virus load in blood (Fig. 4 & 5).
- The fifth passage was cancelled for animal welfare reasons.
- Genome sequencing revealed that gene C257L changed during passaging, suggesting that altered C257L counteracted the ΔI177L-based attenuation leading to reversion to virulence (Table 2).
- A C257L knock-out virus could not be rescued, which suggest that the C257L gene is essential for ASFV

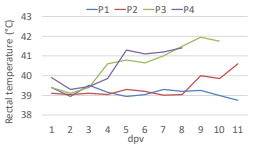

Figure 4. Average daily rectal temperature of pigs in each of the four passages.

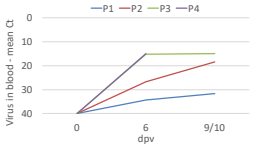

Figure 5. Average virus load as determined by qPCR in blood taken from pigs in each of the four passages.

Table 2. Mutations during passages in three regions of ΔI177L (C257L, B385R and the transgenic region).

	C257L	C257L	C257L	B385R	transgenic
	86865ATTCCTTG86872	87083CATGCAAC87090	87229CGTTTTTTT87237	104906GACGGTC104912	p72-mCherry
P0	Cytosine86868	Guanine87086	7 Thymine	Cytosine104908	No nt changes
P1	C	G	7T	C	
P2	Y (C or T)	G	7T	Y (C or T)	
P3	Y (C or T)	R (G or A)	7T	Y (C or T)	
P4	T (Glu → Lys)	R (G or A)	4T (Lys deleted)	C	

Modified ASFV-G-Δ9GL/ΔUK as a promising vaccine candidate?

- To enhance its safety, ASFV-G-Δ9GL/ΔUK was slightly further attenuated by the deletion of a third gene.
- Provides ≥80% protection against ASFV after 1 shot.
- Is safe for pigs up to titers of $2 \cdot 10^6$ $TCID_{50}$.
- Is genetically stable and does not revert to virulence.
- Very limited virus shedding.
- Does not spread from vaccinated to co-housed naïve pigs.
- Preliminary data showed that it is safe for pregnant sows and their offspring.
- Can be produced on immortalized macrophages (iPAM).

Conclusions

- Vaccine strain ASFV-G-ΔI177L has a severe negative impact on reproductive performance.
- Vaccine strain ASFV-G-ΔI177L is genetically unstable and reverts quickly to virulence.
- A modified version of ASFV-G-Δ9GL/ΔUK addresses the limitation of ΔI177L and shows promise as a vaccine strain.

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[1] MSD Animal Health, Boxmeer, The Netherlands
[2] HUN-REN Veterinary Medical Research Institute (VMRI), Hungária krt. 21, 1143 Budapest, Hungary
[3] Avirtransvac kft., Budapest, Hungary
Correspondence: erwin.van.den.born@merck.com, zadori.zoltan@vmri.hun-ren.hu