Comparison of oral, nasal and anal swabs for detection of African swine fever virus by qPCR in vaccine efficacy studies

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Background

Virus shedding is one of the essential parameters in evaluating African Swine Fever (ASF) vaccine efficacy. For its assessment, oral (OS), nasal (NS), and anal (AS) swabs are typically analyzed for the presence of viral DNA. In a recent multicenter efficacy study conducted at Friedrich Loeffler Institute (FLI), Germany, and at Wageningen Bioveterinary Research (WBVR), Netherlands, within an EU collaboration, live attenuated vaccine candidates were tested for efficacy via oral administration. One of the candidates was also administered intramuscularly as an efficacy control. Challenge was administered oro-nasally. OS, NS and AS were collected both in the vaccination and in the challenge phase, and viral DNA detection was compared across swab routes as well as with blood samples.

Objective

Can a single swab route be reliably used to indicate virus shedding in live animals, thereby eliminating the need to collect and test all three swabs? **This would enhance animal welfare in an experimental setting and would reduce testing costs.**

Materials and methods

Table 1. Study design at both institutes

	# of tested vaccines	Vaccine administration route	# of animals per test group	# of control animals	Challenge administration route
FLI	4	oral	15	5	Oro-nasal
WBVR	3	Oral	10	5	Oro-nasal
	1	IM*	8		
	Days of collected samples vaccination phase			Days of collected samples challenge phase	
FLI	0^, 7, 14, 21, 28			4, 7, 14, 21, postmortem**	
WBVR	0^, 7, 14, 21, 27			2, 6, 9, 14, 21, at HEP***	

* Intramuscular

^Sample from D0 were all negative and were excluded from the analysis

**Postmortem samples from animals at HEP or experiment end were excluded due to blood contamination from epithelial damage caused by the euthanasia method (electric pliers).

*** Humane end point

Blood and swab samples were tested with qPCR using a validated test method. Only swabs collected from live animals were analyzed in this work.

Results

Detection of viral DNA in the vaccination phase

Following oral vaccination, a few blood samples and OS and only 1 NS were positive (Fig. 1a, c). Positive swabs did not always correlate with viremia, suggestive of local virus replication. Viremia also did not always correspond to positivity in swabs. Positive OS were found on 3 out of four sampling days (Fig. 1b, d).

Following IM vaccination, most of the blood samples were positive (Fig. 1e). 10 of those samples correlated with positive OS and with only 1 positive NS. AS remained negative. At least 1 OS was found positive on each sampling day (Fig. 1f).

Detection of viral DNA in the challenge phase

Following challenge, most blood samples were positive (Fig. 1g, i). Larger total number of OS were positive as compared with NS, although the difference was bigger at WBVR. OS were positive earlier than the NS (fig. 1h, j). Also here positive swabs not always correlated with viremia, and viremia not always corresponded to positivity in swabs. Furthermore, at 4 dpc, NS from 7 animals were positive, while the OS were negative (Fig. 1i). The number of positive AS was always the lowest.

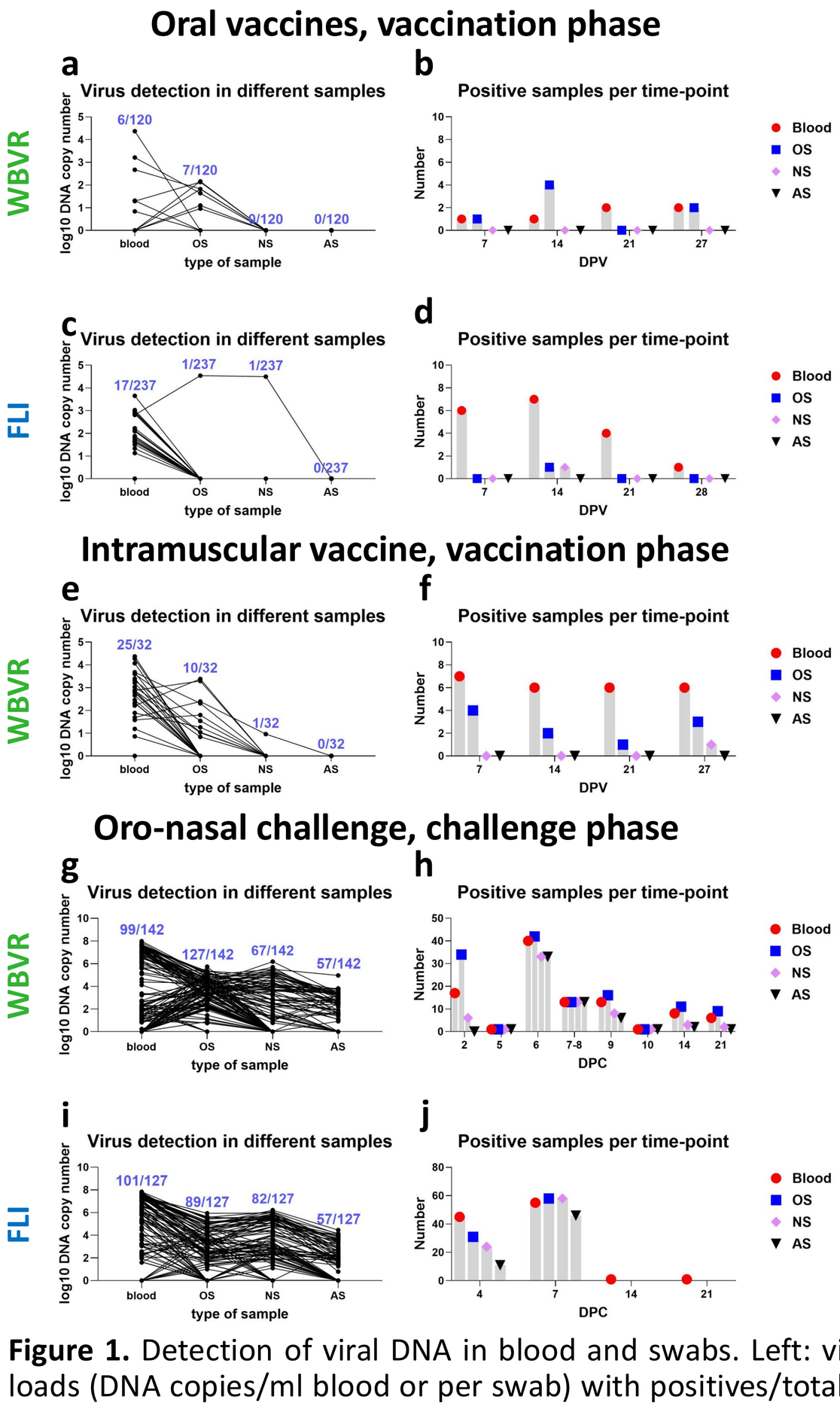

Figure 1. Detection of viral DNA in blood and swabs. Left: viral loads (DNA copies/ml blood or per swab) with positives/total in blue. Right: number of positives per sampling point. DPV – days post vaccination; DPC – days post challenge.

Conclusions

- *OS were the most sensitive for detection of virus shedding during both the vaccination and the challenge phase*
- *Positive swabs did not always correlate with viremia, suggesting local virus replication*
- *Viremia did not always correspond to swab positivity, so swabs cannot replace blood for measuring viremia in the in vivo phase*
- *AS were least sensitive*
- *OS are less invasive than NS for the pigs, and preferred for animal welfare*

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