

Summary: Superficial Inguinal Lymph Nodes for Screening Dead Pigs for African Swine Fever

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Main Points:

- Superficial Inguinal Lymph Nodes (SILNs) samples pose low contamination risk and can be collected quickly without specialized skills
- Evidence shows that SILNs can be used as a reliable sample type for screening for ASFV with some exceptions

African Swine Fever (ASF) is the most critical swine disease found globally. ASF virus (ASFV) causes per-acute and acute disease in domestic and wild pigs and can cause death within two weeks post-infection. ASFV transmission typically occurs through oral or nasal (oro-nasal) contact through direct contact with an infected animal or fomites, or feeding on contaminated feed. The virus replicates in the tonsils and regional lymph nodes, travels through the blood and lymph systems to spread to secondary lymph nodes within 2-3 days. Therefore, the spleen is a good organ for which to test for ASFV but requires the carcass to be opened to obtain the sample. Opening a carcass creates the risk of environmental contamination and subsequent spread of ASFV. Alternative OIE recommended sample types (lymph nodes, bone marrow, lung, tonsil, and kidney) require a full necropsy, highly skilled staff, special instrumentation, and is time-consuming. For this research, superficial inguinal lymph nodes (SILNs) were being proposed as a sample type to test for ASFV because they are easy and fast to collect with minimal environmental contamination and do not necessitate skilled labor.

In all experiments, 4–5 week-old weaned pigs were used. A series of 6 experiments were done with oro-nasal, intramuscular, or indirect infections (through seeder pigs) using 5 ASFV strains (Estonia 2014, Ghana 20, Nigeria RV502, Vietnam2561, and Georgia 2007/1). All animals that were euthanized, were done at humane endpoints (between days 7-22). All pigs at the time of death or euthanasia had their SILNs and spleen samples collected. ASFV genomic DNA was detected using a quantitative real-time PCR. The Pearson correlation coefficient between the ASFV genomic copy numbers from the spleen and SILNs was calculated.

Clinical signs for pigs varied by ASFV strain type but included fever, lethargy, hematochezia, and rectal bleeding. When the ASFV genome log copy numbers in the spleens were compared with those in the SILNs, there was a positive correlation ($r > 0.7$, $p < 0.0001$) for all moderately virulent (Estonia 2014) and highly virulent (Ghana 20, Nigeria RV502, Vietnam2561, Georgia 2007/1) ASFV strains considered collectively, the amount of virus in spleen samples were positively correlated with the SILN samples ($r = 0.77$) with high confidence ($p < 0.0001$). For just moderately virulent strains $r = 0.85$ and just for highly virulent strains $r = 0.70$. Regardless of the ASF strain, SILNs showed highly correlated genomic copy numbers to those in the spleen, indicating that they are a suitable sample type for ASFV detection.

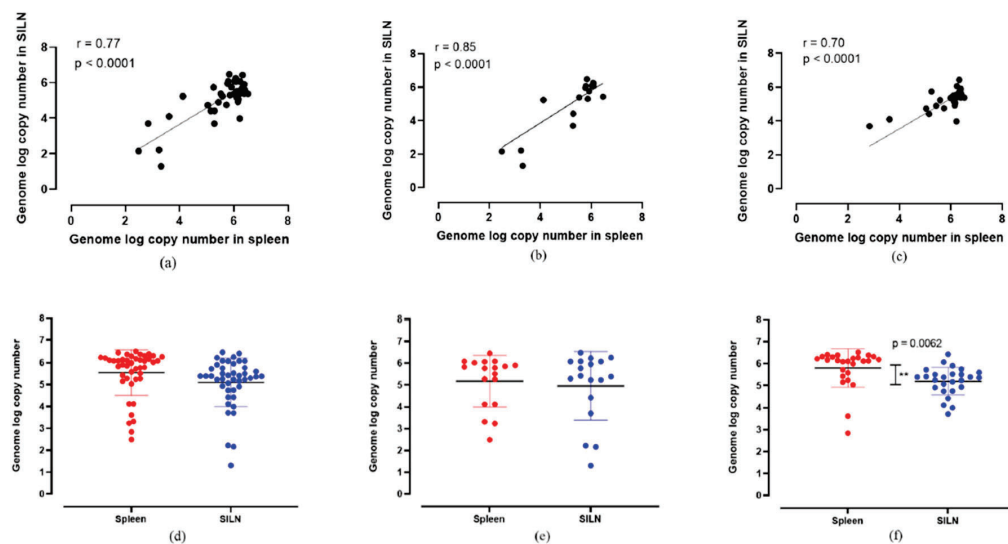


Figure 1: Correlation of ASFV genome log copy numbers detected between spleen and SILNs together with the scatter dot plots indicating the relative distribution of detection levels in all ASFV strains (a,d), ASFV Estonia only (b,e), and highly virulent strains (c,f). The ASFV genome log copy numbers detected in spleens and SILNs correlated positively ($r = 0.77$, $r = 0.85$, and $r = 0.70$) at statistically significant levels ($p < 0.0001$). When the unpaired t-test was performed, there was no significant difference between mean genome log copy numbers, except among the highly virulent strains, where the mean genome log copy numbers were significantly different $** (p = 0.0062)$.

This study shows initial evidence that SILNs can be used as a reliable sample type for rapid screening of dead pigs for ASF when a complete necropsy is not possible or not desirable. The ability to use SILNs for screening dead pigs may enable both ASF-free, as well as ASF-endemic countries, to streamline and expand their passive surveillance for ASF. Even though the data presented in this study are from domestic pigs, the sampling method is equally beneficial and applicable to ASF surveillance of wild pigs. A consideration for when to use this method is that testing based on SILNs is not suitable for routine disease investigations in domestic pigs where other infectious agents such as PRRSV, PCV2, or bacterial infections are suspected.

The full paper is open access and can be found at: <https://doi.org/10.3390/v14010083>