MOLECULAR PROFILING OF CYTOKINE GENE EXPRESSION: INSIGHTS INTO THE IMMUNE RESPONSE OF PIGS INFECTED WITH LOCAL ISOLATES OF ASFV IN THE PHILIPPINES

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1 Introduction

African Swine Fever (ASF) has resulted in widespread outbreaks and reductions in hog production in the Philippines since 2019. To control its spread, ASF vaccine trials are being carried out in the country; however, no vaccine has been approved for commercial use yet.

The continued prevalence of this disease is partly attributed to limited understanding of the immune response to its causative agent, African Swine Fever Virus (ASFV), particularly in relation to local virus isolates. The ASFV is known to elicit strong immunological response, which lead to dysregulation of cytokines, the important mediators that control immune cell activation and mobilization.

This study aims to elucidate host-pathogen interactions by profiling the gene expression of immune-related cytokines, chemokines, and innate cell receptors in ASFV-infected pigs in the Philippines. Understanding these could further the knowledge of ASFV pathogenesis aimed at developing effective immunological interventions to manage and prevent ASF.

Summary:

- Profiling of immune gene expression in ASFV-infected pigs in the Philippines.
- Understanding host-pathogen interactions in locally infected pigs.

2 Methods/Approach

Collection of 25 ASF(+) and one ASF(-) whole blood sample from provincial veterinary offices in the Philippines → RNA extraction using Zymo™ Quick-RNA Miniprep Kit (Cat. No. R1075) → Reverse transcription quantitative polymerase chain reaction (RT-qPCR) of immune-related genes → Fold change calculation using $2^{-\Delta\Delta Ct}$

Target immune-related genes

IL-1β	IL-17A	IL-33	CCL3L1	CXCL8
IL-5	IL-18	IFN-γ	CCL4	CXCL10
IL-6	IL-21	TNF-α	CCL5	TLR2
IL-13	IL-23	CCL2	CXCL2	TLR3

Reference gene: β-actin

Data analysis: Data normality was assessed using Shapiro-Wilk test and homogeneity of variances was evaluated using Levene's test. Wilcoxon-signed rank test was used to to determine whether the change in the expression of each target gene is significant.

3 Results

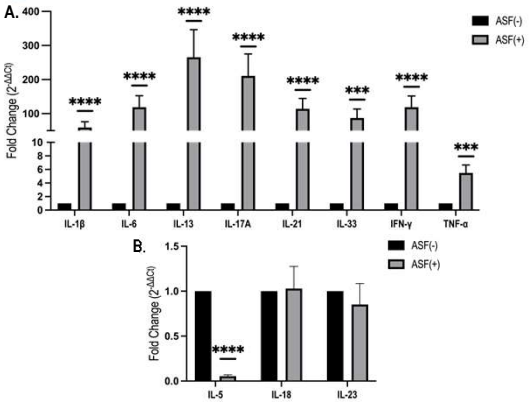

Figure 1. Cytokine expression levels in ASF(+) whole blood samples compared to ASF(-) samples (Mean ± SEM; Wilcoxon-signed rank test; ***p ≤ 0.001, **p ≤ 0.0001).

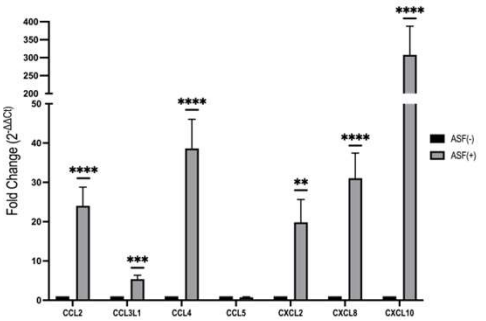

Figure 2. Chemokine expression in ASF(+) whole blood samples compared to ASF(-) samples (Mean ± SEM; Wilcoxon-signed rank test; **p ≤ 0.01, ***p≤0.001; ****p ≤ 0.0001).

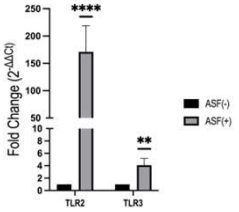

Figure 3. Toll-like receptor (TLR) expression in ASF(+) whole blood compared to ASF(-) sample (Mean ± SEM; Wilcoxon-signed rank test; **p ≤ 0.01, ****p ≤ 0.0001).

4 Discussion

- Upregulation of pro-inflammatory cytokines IL1-β, IL-6, IL-13, IL-17A, IL-21, IL-33, IFN-γ, and TNF-α (Figure 1A) agrees with previous findings, indicating a strong immune response and potential cytokine storm.
- In contrary, IL-5 was downregulated, while IL-18 and IL-23 were unchanged (Figure 1B), highlighting host dynamic immune profile.
- Upregulation of all chemokines, except CCL5, suggests active immune cell recruitment and inflammation (Figure 2). Notably, CCL5 expression was unchanged—contrary to previous reports—implying possible ASFV immune evasion mechanism.
- Lastly, upregulation of TLR2 and TLR3 (Figure 3) indicates activation of innate immune recognition pathways.

5 Conclusion

Overall, this study highlights a robust but potentially dysregulated immune response involving cytokine upregulation, chemokine-driven cell recruitment, and innate immune activation in pigs locally infected with ASFV in the Philippines.

This knowledge further our understanding of the host immune response and may be the foundation for further studies aimed at developing effective immunological interventions to manage and prevent ASF.

6 References & Aknowledgements

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