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Rethinking ASF control: Can we halt the ASF cytokine storm and save pigs

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Abstract

African swine fever (ASF) remains a global threat to the pig industry, with high mortality rates and limited control options due to the absence of effective vaccines or treatments. This review proposes a therapeutic framework to manage ASF, targeting the excessive immune response known as the "cytokine storm," which leads to severe organ damage and death. Based on ASF progression timelines, fever typically appears 3-4 days into the infection. At this critical point targeted therapies such as anti-inflammatory agents could be administered at this critical stage to constrain the initial generation of cytokines in an attempt to delay a full blown cytokine storm. However, long-term use of anti-infammatories could dampen the pig's immune response and so would need to be managed carefully. There is increased multi-organ damage leading to blood vessel permeability causing bleeding, which calls for blood anticoagulants to be used to prevent complications such as the blood clotting and fluid accumulation in vital organs such as in the lungs. The proposed therapeutic approach, synchronized with ASF progression, could help reduce mortality by modulating the immune response and managing complications.

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Introduction

African Swine Fever (ASF) is a highly destructive viral disease that affects both domestic and wild pigs, resulting in cataclysmic losses for the global swine industry (Galindo and Alonso, 2017). Caused by the African Swine Fever Virus (ASFV) from the Asfarviridae family, ASF can lead to mortality rates as high as 100% in some outbreaks, posing a significant threat to food security and economic stability in affected regions (Blome *et al.*, 2013; Li *et al.*, 2022). Control measures for ASF have concentrated around stricter biosecurity measures, farmer sensitization, culling of infected animals and vaccine development. These current control measures have proven insufficient to control the spread of ASF (Liu *et al.*, 2021; Klein *et al.*, 2024).

Prevention measures could include animal movement controls, improved farm sanitation and a need for comprehensive surveillance networks (Urbano and Ferreira, 2022; Wang *et al.*, 2024). Effective biosecurity is a critical tool in preventing ASF spread, as Loeffen *et al.* (2021) emphasize that this is very important and specifically useful for smallholder farming, which may be resource constrained. Public awareness and education crusades have also gained attention as vital components of ASF control strategies. By educating farmers and stakeholders about transmission routes and effective control measures, these initiatives aim to reduce the risk of human-mediated spread through contaminated meat products. Increased awareness and compensation in case of losses are critical for fostering community engagement in biosecurity practices (Dixon *et al.*, 2017). There are efforts to control ASF through development of vaccines against ASF globally. Several studies have indicated the potential of different vaccines including live attenuated vaccines, subunit vaccines, and DNA vaccines (Gavier-Widén *et al.*, 2020; Liu *et al.*, 2021; Zhou *et al.*, 2022; Diep *et al.*, 2024; Wang *et al.*, 2024). One of the most pressing challenges is the lack of a commercially available vaccine that provides comprehensive protection and cross protection against all ASFV strains. The genetic diversity of ASFV complicates vaccine development, as different strains exhibit significant variations in virulence and immune response (Galindo *et al.*, 2008; Wang *et al.*, 2024). The absence of effective vaccines or antiviral treatments further complicates containment efforts (Fernández-Pinero *et al.*, 2013; Diep *et al.*, 2024), highlighting the urgent need for alternative disease management strategies. The complexity of ASFV, its poorly understood antigenic epitopes, and unclear protective mechanisms have hindered vaccine development. While past attempts with inactivated, DNA, and subunit vaccines have largely failed, live-attenuated virus vaccines (LAVs) have shown promise, though challenges remain to optimize their efficacy and safety (Monteagudo *et al.*, 2017; O'Donnell *et al.*, 2017; Reis *et al.*, 2017; Borca *et al.*, 2020; Gavier-Widén *et al.*, 2020; Liu *et al.*, 2021; Diep *et al.*, 2024). In the absence of effective vaccines, ASF management relies heavily on stringent biosecurity, animal culling, and movement restrictions (Liu *et al.*, 2021). However, these measures alone have not been sufficient to stop the spread of ASF, leading to widespread economic damage and significant disruptions in pig farming operations.

Given the limitations of current control strategies, exploring alternative solutions, including therapeutic interventions, becomes essential. While several compounds have been identified with anti-ASFV activity in vitro, including aUY11, fluoroquinolones, resveratrol, oxidized resveratrol, and amiloride (Liu *et al.*, 2021), their effectiveness in live ASFV-infected pigs remains unproven. More research is needed to determine their potential as viable treatments in clinical settings. This mini-review highlights the potential of targeting the "cytokine storm," a hyper-inflammatory response that plays a central role in ASF pathology. Parallels can be drawn to treatments used in human diseases such as COVID-19, where managing the cytokine storm was key to reducing mortality. For farmers with valuable breeding stock or individual pigs of high economic importance, investing in therapeutic treatments could be a rational choice, especially if the cost of treatment is less than the potential financial loss.

Developing and validating such therapeutic approaches could provide farmers with new options for ASF management beyond current biosecurity measures and culling practices.

Current ASF management: Strengths and limitations

Traditionally, ASF management has relied on strict biosecurity measures, movement restrictions, and culling of infected animals (Liu *et al.*, 2021; Klein *et al.*, 2024). While these measures have to some extent been successful in controlling outbreaks, their limitations are clear. In resource-poor regions, where biosecurity is hard to enforce, the disease continues to spread. The absence of an effective ASF vaccine further complicates control efforts, despite ongoing research into live-attenuated virus (LAV) vaccines (Gavier-Widén *et al.*, 2020). Additionally, antiviral compounds such as fluoroquinolones and resveratrol have shown some promise in laboratory settings, but these have not yet been translated into successful in vivo treatments for ASFV (Liu *et al.*, 2021). Given the complexity of the virus and its ability to evade the immune system, innovative therapeutic approaches are needed to complement existing biosecurity measures.

The cytokine burst and storm in ASF pathogenesis

In recent years, cytokines have gained significant importance as diagnostic, prognostic, and therapeutic agents in both human and animal diseases (Dinarello, 2007; Dawson *et al.*, 2020). One of the most significant challenges in managing ASF is the uncontrolled immune response known as a "cytokine burst" or "cytokine storm." Upon infection with ASFV, the host pig's immune system releases an excessive number of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IFN-α, leading to widespread tissue damage, increased vascular permeability, and multi-organ failure (Wang *et al.*, 2021). This hyper-inflammatory response often results in the pig's death within days of infection, as the cytokine storm severely compromises the animal's immune system. In human medicine, particularly during the COVID-19 pandemic, managing the cytokine storm was key to reducing severe disease outcomes (Channappanavar and Perlman, 2017; Song *et al.*, 2020; Montazersaheb *et al.*, 2022). Anti-inflammatory treatments, blood thinners, and antibiotics were used to modulate the immune response and manage the complications arising from excessive cytokine production (Mehta *et al.*, 2020; Chen *et al.*, 2021). Applying similar principles to ASF management could offer a novel approach to reducing mortality in infected pigs, especially in high-value breeding stock.

Therapeutic approaches for ASF: Lessons from COVID-19

As form many viral infections, current research towards controlling and containing ASF is focused on preventive approaches with little or no attention paid to other approaches including therapeutic management of the disease (Liu *et al.*, 2021; Urbano *et al.*, 2022). As the long walk towards vaccine development continues there is need to have quicker ways of damage control. We saw this in the management of SAS-COV diseases in recent years including remedies for management of COVID-19 and one wonder if the approaches deployed during covid-19 could be applied in bridging the gap in controlling ASF (Pakotiprapha *et al.*, 2023). Combinations of drugs including anti-inflammatories, antibiotics and anticoagulants were tested and proven to be effective in managing the severe disease's symptoms leading to recovery of a significant number of human patients (Langarizadeh *et al.*, 2021; Zhou *et al.*, 2020; Consolaro *et al.*, 2022).

Anti-inflammatory agents

In COVID-19 treatment, anti-inflammatory drugs such as corticosteroids (e.g., dexamethasone) were used to suppress the overactive immune response and prevent the escalation of the cytokine storm (Stockman *et al.*, 2006; Langarizadeh *et al.*, 2021; Zhou *et al.*, 2020; Consolaro *et al.*, 2022). In ASF-infected pigs, a similar approach could help to reduce the inflammation and tissue damage caused by excessive cytokine production. Careful dosing would be required to avoid weakening the immune system's

ability to fight ASFV, but early intervention with anti-inflammatory agents could potentially mitigate the severe symptoms associated with ASF.

Antibiotics

Though ASFV is a viral infection, secondary bacterial infections can complicate the disease, especially in pigs with weakened immune systems. As seen in COVID-19, antibiotics were used to treat secondary bacterial infections that occurred as a result of the immune system's dysfunction (Russell *et al.*, 2020; Consolaro *et al.*, 2022; Reese *et al.*, 2022). The use of broad-spectrum antibiotics could therefore be considered in managing ASF cases, especially where bacterial complications are suspected. However, it is crucial to use antibiotics judiciously to prevent antimicrobial resistance.

Blood anticoagulants

Another lesson from COVID-19 management is the use of blood thinners (anticoagulants) to prevent complications such as blood clotting, which is often triggered by excessive inflammation. As in humans (Jarczak and Nierhaus, 2022; Mohseni *et al.*, 2023), in ASF, the cytokine storm similarly leads to vascular damage, which may contribute to haemorrhage, clot formation and organ failure (Wang *et al.*, 2021). Administering anticoagulants in ASF-infected pigs could help prevent thrombosis and improve survival rates by preserving organ function during the disease's acute phase.

Immunomodulatory therapies

Immunomodulatory drugs offer another potential avenue for ASF treatment. These therapies aim to modulate the immune responses, preventing it from becoming overactive while still allowing the body to mount an effective defence against the virus (Afe *et al.*, 2023). For example, drugs targeting specific cytokines like IL-6 or TNF-α—used in conditions such as rheumatoid arthritis and COVID-19—could help reduce the severity of the cytokine storm in ASF-infected pigs (Mehta *et al.*, 2020). The challenge lies in finding the right balance between dampening the immune response and preserving the pig's ability to combat the virus.

Supportive care and other therapies

Supportive care, such as fluid therapy and nutritional support, plays a vital role in managing diseases with severe systemic effects, like ASF. These interventions aim to maintain homeostasis, support organ function, and buy time for the immune system to recover (Hoste *et al.*, 2014). In combination with pharmacological treatments, supportive care could help reduce ASF mortality, especially in farms where individual pigs or breeding stock are of high value. As demonstrated in COVID-19, ensuring adequate hydration, nutrition, and organ support can significantly improve patient outcomes.

Proposed ASF therapeutic management framework

African Swine Fever (ASF) is marked by a dysregulated immune response that triggers a cytokine cascade (burst) with an excessive release of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IFN-α, resulting in a "cytokine storm" that causes severe tissue damage, vascular leakage, multi-organ failure, and death (Oura *et al.*, 1998; Wang *et al.*, 2020; Franzon *et al.*, 2023). Drawing from successful therapeutic strategies used in human diseases with potential cytokine storms like COVID-19, a timeline-based approach is proposed for managing ASF by targeting the cytokine storm (Polak *et al.*, 2020; Salasc *et al.*, 2022; Gonçalves *et al.*, 2020). This approach focuses on appearance and treatment of clinical signs along the disease's progression timeline (Fig. 1 and 2). The approach is hinged on three cardinal pillars including, 1. the use of anti-inflammatory agents, 2. the use of antibiotics and 3. the use of blood anticoagulants/thinners. On the third day of ASF infection, when fever typically emerges (Fig. 2) (Wang *et al.*, 2020), corticosteroids like dexamethasone could be administered to suppress the increasing inflammatory response and prevent the cytokine burst from developing into a full-blown storm. However, prolonged use of corticosteroids can compromise the immune system, increasing susceptibility to secondary infections, thus, broad-spectrum antibiotics may also be necessary to combat secondary bacterial infections arising from the weakened immune system, improving survival chances.

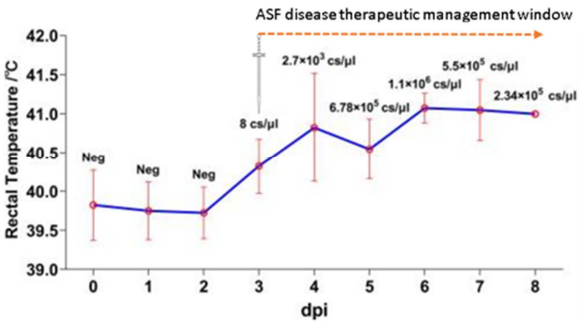

Fig. 1. Kinetics of rectal temperatures. Animals were inoculated with 1,000 FFU (1 mL) of African swine fever virus (ASFV) SY18 intramuscularly. Rectal temperature and clinical signs were monitored daily. Data are shown as mean (SD). Viremia was analyzed through quantitative PCR (qPCR) method. The virus titers in blood were quantified by calculating the concentrations of ASFV genome copies via a standard curve based on serial diluted plasmids bearing the B646L gene. Values (copies/μL) are shown above the points. (Adapted from Wang *et al.*, 2020)

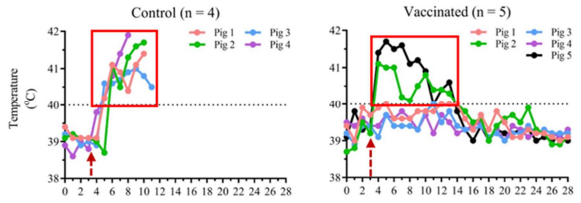

Fig. 2. Protection efficacy of HLJ/18-7GD in pigs challenged with the prevalent highly virulent genotype II variant HuB/628/20. HLJ/18-7GD-vaccinated and control pigs were challenged with 101.5 HAD50 of HuB/628/20, and were monitored daily for rectal temperature (A and B) for 28 days post-challenge. The dashed black lines in panel A indicate the threshold of normal rectal temperature. (Adapted from Wang *et al.*, 2024)

As the disease progresses and haemorrhages occur, anticoagulants can be employed to control internal bleeding caused by increased vascular permeability, particularly in the lungs, preventing fluid accumulation and respiratory failure. Although this therapeutic framework is promising, it will require further research and clinical trials to optimize treatment regimens and address challenges such as rapid diagnosis, cost-effectiveness, and judicious antibiotic use to prevent antimicrobial resistance. Integrating this treatment approach with current biosecurity measures and on-going vaccine development offers the most promising strategy for reducing ASF mortality and preserving valuable pig populations.

Conclusion

African swine fever remains a significant threat to the global swine industry, with existing management strategies proving inadequate to fully control the disease. As we rethink ASF management, the use of therapeutic interventions, particularly those targeting the cytokine storm, presents a promising avenue for reducing mortality. Drawing on lessons from the COVID-19 pandemic, where anti-inflammatory drugs, antibiotics, and blood thinners were used successfully to manage severe disease, similar strategies could be applied to ASF. By modulating the immune response and providing supportive care, it may be possible to improve survival rates and preserve valuable pig populations. Further research and trials will be needed to optimize this strategy, complementing biosecurity and vaccine development efforts to enhance ASF control and safeguard pig populations.

Challenges and future directions

Implementing therapeutic interventions for ASF faces several challenge, including cost, scalability, and the need for timely intervention. Developing countries, where ASF outbreaks are more frequent, may find it difficult to implement complex and costly treatments like immunomodulators and blood thinners. Additionally, it is critical to administer these therapies early in the disease course following a timeline before irreversible organ damage occurs. Research into the molecular mechanisms driving the cytokine storm in ASF is still in its infancy. Understanding these mechanisms more thoroughly will help identify new drug targets and refine treatment strategies. Clinical trials of anti-inflammatory drugs, antibiotics, and blood thinners in pigs could provide valuable data on the efficacy of these therapies in reducing ASF mortality. The integration of these therapeutic strategies with

biosecurity measures and ongoing vaccine research could revolutionize ASF management in the coming years.

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