

## Evaluating on-farm sampling strategies for early detection of African swine fever

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### Key Points:

- Sampling many pens (e.g., 30) with one pig per pen increases the probability of detecting ASF.
- Sampling few pens (e.g., 5) with many pigs per pen (e.g., 6) reduces the probability of detection, especially when other diseases are present

### Introduction

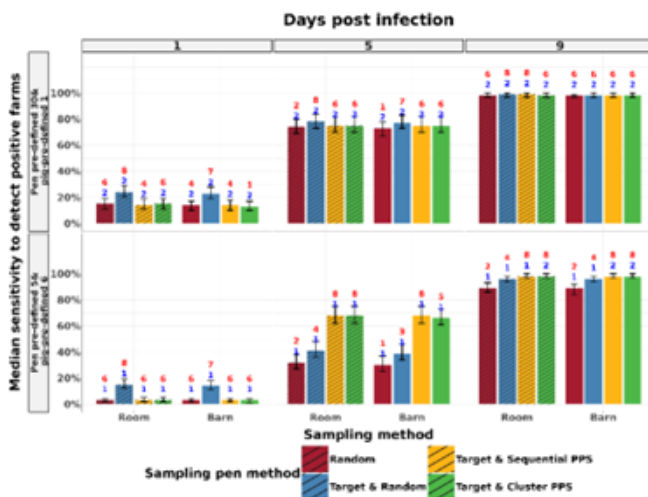
Early detection of African swine fever virus (ASFV) is critical to prevent large-scale outbreaks and maintain business continuity in the U.S. swine industry. Detecting ASFV at the farm level is challenging during the early stages of infection, when prevalence is low, animals may be asymptomatic, and clinical signs can be confused with endemic diseases such as PRRSV. Current surveillance approaches lack clear guidance on how many pens and animals should be sampled, creating uncertainty in outbreak response. In this study, we systematically assess multiple sampling strategies for detecting ASFV-positive animals on suspected farms using simulated outbreak scenarios. The analysis includes high- and moderate-virulence ASFV strains, the presence of PRRSV as a co-circulating disease, four pen-level sampling approaches, and variations in the number of pigs and pens sampled.

### Methods

A high-resolution stochastic simulation model was applied to 1,865 commercial swine farms across 33 U.S. states. 1) The study evaluated: High- and moderate-virulence ASFV strains, 2) The presence of PRRSV as a co-circulating disease, 3) Four pen-selection strategies, including random, clinical (targeted), Informative sequential and informative cluster sampling, 4) Multiple combinations of number of pens and pigs sampled per pen. Sampling performance was measured as the probability of detecting at least one ASFV-positive animal per farm within the first 14 days after virus introduction.

### Results

Detection probability was primarily driven by the number of pens sampled rather than the number of pigs sampled within each pen. Strategies that maximized spatial coverage, such as sampling 30 pens with one pig per pen, consistently achieved the highest detection sensitivity across epidemiological scenarios (Fig. 1). In contrast, sampling five pens with six pigs per pen resulted in substantially lower detection probabilities (Fig. 1), with even lower probabilities when clinical signs were unreliable due to a high prevalence of co-circulating PRRSV. Among pen-selection approaches, the combined target and random strategy outperformed informative strategies when 30 pens were sampled. Additionally, ASFV virulence influenced detection performance, with high-virulence strains being detected more rapidly and consistently than moderate-virulence strains.



**Figure 1.** Sensitivity analysis of high virulence ASFV detection through the utilization of diverse pen sampling and animal sampling methods at both barn and room levels, observed over a sequential series of days post-infection (DPI). Sampling strategies are represented by a unique color code: Random (red), target & cluster (green), target & random (blue), and target & sequential (yellow). Sensitivity metrics are calculated by identifying the presence of pigs in any ASFV states Exposed, Clinical, Sub-Clinical, Carrier, PRRSV-Exposed, and PRRSV-Clinical within each epidemiological unit. Numerical rankings in descending order are depicted in red and blue to indicate the effectiveness of sampling methods. Red numbers represent the rankings (from best 8 to least effective 1) of pen sampling methods across different room and barn levels within each combined DPI and sample size schema. Blue numbers indicate the highest-ranked methods on each specific DPI for each sampling pen method (from best 2 to least effective 1). Methods with identical median sensitivity values were assigned the same rank, using the upper rank value.

### Discussion

To optimize farm-level ASFV detection while limiting the number of animals sampled, the most effective approach was to collect samples from one pig per pen across 30 pens, or all pens when fewer were available. When this strategy was combined with targeted sampling of pens exhibiting clinical signs, supplemented by random selection when signs were absent, it achieved a median detection sensitivity of 29% by four days post-introduction (DPI), increasing to 93% thereafter. This strong performance is driven by within-barn transmission dynamics, as ASFV tends to spread more rapidly within pens than between pens. Consequently, pigs sharing a pen with an infected animal are more likely to become exposed earlier than those in adjacent pens. Under early outbreak conditions, when only a small number of pens are infected and clinical signs may not yet be evident, increasing the number of pens sampled enhances the likelihood of identifying infected animals. Overall, these findings provide quantitative support for improving surveillance guidelines and strengthening preparedness and response strategies for ASF and other transboundary animal diseases.

This work was supported by the Foundation for Food & Agriculture Research (FFAR) award number [FF-NIA21-000000064](https://www.fffar.org/award/FF-NIA21-000000064) and from the National Institute of Food and Agriculture, Data Science for Food and Agricultural Systems (DSFAS), A Novel Multilevel Model of Swine Disease Spread to Assess the Effectiveness and Feasibility of African Swine Fever Control and Eradication Strategies, award number [2024-67021-43841](https://www.nifa.gov/award/2024-67021-43841), from the U.S. Department of Agriculture's National Institute of Food and Agriculture.

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