Field comparison of ASFV target tissues and non-invasive samples in wild boars during the genotype II Italian epidemic

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1 Introduction

African swine fever (ASF) is a highly lethal viral disease affecting domestic and wild pigs, caused by a dsDNA virus (*Asfarviridae* family, *Asfivirus* genus). Virus detection typically targets organs such as the spleen, kidneys, lungs, tonsils, lymph nodes, and bone marrow from deceased animals. Field collection of such tissues may result in environmental contamination. Currently, the official method for ASF diagnosis in Italy involves collecting the spleen from dead wild boars, a procedure that requires opening the abdominal cavity and can carry a high risk of contamination. Non-invasive methods, such as collecting biological samples from feces, blood, or nasal swabs, could serve as a valid and safer alternative. The introduction of non-invasive diagnostic techniques could therefore improve the effectiveness of ASF monitoring in wild boars and contribute to a safer and more sustainable management of the disease in endemic areas.
Recent studies based on experimental infections[1,2,3,4,5,6] have focused on non-invasive (NI) samples (faeces, blood, oral, and nasal swabs) for easier field collection and faster diagnosis.

The **aim of this study** was to compare ASFV detection in target organs (spleen and kidney) and non-invasive (NI) samples from wild boars during passive surveillance in an endemic area in North-West Italy in 2023-2024.

Key Points:

- **Current diagnostic methods include target organs** such as spleen, kidney, lungs, tonsils, lymph nodes, and bone marrow.
- **Non-invasive surveillance might be implemented** using feces, oral, and nasal swabs to reduce environmental contamination.

2 Materials & Methods

In the restricted zones of the current ASF outbreak in the Liguria-Piedmont regions, alternative biological matrices were sampled between November 2023 and June 2024 (Fig. 1a). Oral and nasal fluids were collected using sterile swabs, while faeces were collected from the rectal ampoule and stored at refrigeration temperature (Figg. 1b-1c). These samples were collected alongside standard spleen and kidney samples for comparative analysis. Molecular analysis was performed using Real-Time PCR, with DNA extracted through manual or automated methods using commercial kits. This procedure was applied to both target (spleen, kidney) and alternative (faeces, oral, nasal fluids) matrices, with possible adjustments. Swabs were initially suspended and vortexed in a 1.5 ml volume of MEM with 1% antibiotics, and subsequently, 300 µl of the suspension was withdrawn for the extraction procedure. The extracts were analyzed to verify the presence of ASF DNA using the ID Gene™ African Swine Fever Duplex amplification kit.
The PCR cycling parameters consisted of a series of steps with alternating temperature cycles: 95°C for 2 minutes followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds.
Sensitivity (SE) and specificity (SP) of the NI tests were estimated using target organ testing as the gold standard (a boar was considered ASFV positive if any organ tested positive). The agreement between NI samples and the gold standard was evaluated using Gwet's AC1.

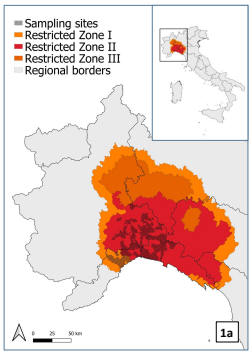

Figure 1. (a) Sampling sites and ASF restricted zones; (b, c) In-field collection of the spleen from a wild boar.

3 Results

NI samples were collected along with target organs from 172 dead wild boars, resulting in 1,017 samples processed by Real-Time PCR. Sixty-six (38%) wild boars tested positive for ASFV in target organs, and sixty-eight (40%) had at least one positive NI sample. Sixty-two (36%) wild boars showed Ct values below 30 in at least one NI sample, with nasal swabs generally having lower Ct values than other NI matrices. Two wild boars with negative target organs had at least one positive NI sample. The estimated sensitivity, specificity, and Gwet's AC1 values are reported in Figures 2 and 3.

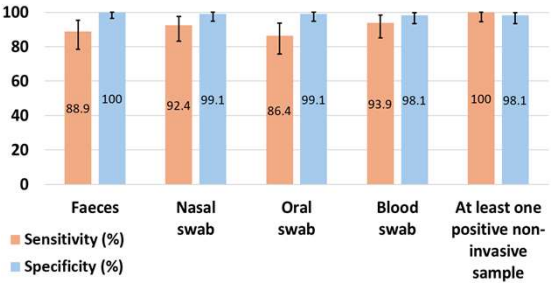

Figure 2. Estimated sensitivity and specificity with 95% confidence intervals

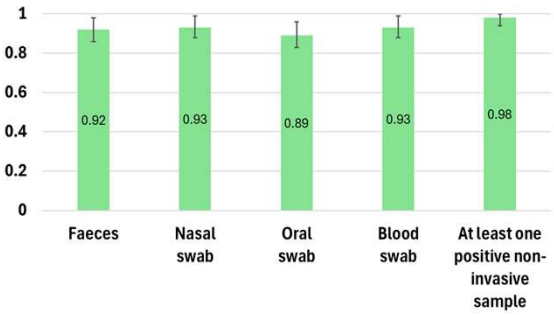

Figure 3. Gwet's AC1 with 95% confidence intervals.

4 Discussion & Conclusions

This is the first field comparison of NI samples and target organs for the detection of ASFV in wild boars. Tests conducted on NI samples (faeces, nasal and oral swabs) showed high sensitivity and specificity and a very good agreement with the gold standard (Gwet's AC1>0.81). Among NI matrices, nasal swabs showed the highest sensitivity (92,4%), while all NI matrices exhibited specificity >98%.
The results of this study highlight the promising potential of NI diagnostic methods for detecting ASFV in wild boars, particularly in field settings. This suggests that NI samples – particularly nasal swabs – could serve as viable alternatives to the traditional method of using target organs for ASFV detection in wild boars, although further validation studies on additional wild boar populations are necessary.

First field comparison: non-invasive samples (nasal/blood swabs) vs. target organs for ASFV detection.
High detection rate: 40% of wild boars tested positive using non-invasive samples, comparable to organs.
High sensitivity: nasal swabs showed 92.4% sensitivity, proving reliable for ASFV detection.

5 References & Aknowledgements

1. Niederwerder et al. 2021
2. Lee et al. 2021
3. Flannery et al. 2020
4. Davies et al. 2015
5. Guinat et al. 2014
6. de Carvalho Ferreira et al. 2014

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Slide 1

AF0 Aggiungere affiliazione?
Arianna Fulmini, 2025-04-07T13:12:43.759

BM0 0 aggiunte
Moroni Barbara, 2025-04-15T13:08:31.583

MB1 Non c'è il dato di digital
Maria Serena Beato, 2025-04-08T13:15:50.710

MB1 0 Se non lo si mette toglierei la frase
Maria Serena Beato, 2025-04-08T13:27:12.222

BM1 1 Ok l'ho tolto, perché ancora stanno analizzando quindi non metterei nulla
Moroni Barbara, 2025-04-15T12:52:56.217

MB2 Indicare quali, e indicare il periodo dello studio, si potrebbe anche inserire una mappa
Maria Serena Beato, 2025-04-08T13:19:52.235

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