

Identification of diagnostic methods for African swine fever: A systematic literature review



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ABSTRACT

This review emphasizes the urgent need for effective diagnostic strategies for African swine fever (ASF), a serious disease affecting pig populations worldwide. The aim of the review is to analyze the existing research on ASF diagnostics through a comprehensive literature review, focusing on different diagnostic approaches, including clinical assessments, PCR tests, ELISA, rapid tests and epidemiological models. It examines their sensitivity, specificity and overall performance, addressing challenges such as the varying sensitivity of tests and cross-reactivity. The review highlights both the strengths and limitations of current methods and suggests areas for improvement and standardization. Recommendations are made for future research and the development of innovative diagnostic tools to improve ASF surveillance and control. This study makes a practical contribution by providing a detailed assessment of ASF diagnostic methods from which veterinary scientists and practitioners can benefit. Theoretical contributions include the identification of gaps in ASF diagnostics and the refinement of discussions on diagnostic accuracy and reliability. These findings are consistent with the journal's focus on infectious diseases and veterinary research and support progress in veterinary medicine and animal welfare.

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Introduction

African swine fever (ASF) is a highly contagious viral disease that severely affects both domestic and wild pigs and poses a major threat to the global pig industry (Gallardo, Fernández-Pinero & Arias, 2019; Dixon, Stahl, Jori, & Pfeiffe, 2020; Pikalo et al., 2021). The disease is caused by African swine fever virus (ASFV), a large DNA virus from the Asfarviridae family. ASF leads to a high mortality rate in infected pigs, triggers trade restrictions and requires costly control measures, which significantly affects the economy (Gallardo et al., 2019; Pikalo et al., 2021). Timely and accurate diagnosis is crucial for controlling outbreaks. Although conventional diagnostic methods such as virus isolation, serology and histopathology have been used, they have limitations in terms of sensitivity, specificity and speed (Dixon et al., 2020).

Recent advances in molecular techniques, particularly polymerase chain reaction (PCR), have transformed ASF diagnostics by enabling rapid and specific detection of ASFV genetic material. Additional methods such as loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA) and antigen detection assays have also emerged, promising to improve speed and accuracy, especially in resource-limited settings (Shi et al., 2024). However, debates remain about the comparative performance of these methods under different environmental conditions and with different viral strains.

Key publications have highlighted the ongoing challenges in ASF diagnosis and the need for diagnostic tools that are reliable and adaptable for use in the field. Systematic literature reviews (SLRs) are increasingly being used to provide a comprehensive, unbiased

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assessment of existing research. SLRs provide a structured approach to summarize the strengths, limitations and gaps of current diagnostic methods, making them ideal for comparing the accuracy and reliability of different techniques. By minimizing bias through predefined selection criteria, the SLR helps to evaluate complex topics such as ASF diagnostics and serves as a basis for future research and practical applications.

Research questions

- i. What published literature is available on diagnostic methods for African swine fever (ASF), including traditional and molecular techniques such as virus isolation, serology, PCR, LAMP, RPA and antigen detection assays?
- ii. What diagnostic performance metrics (sensitivity, specificity), sample types, ASFV strains and environmental conditions are reported in the identified literature?
- iii. How do the different diagnostic methods for ASF perform in terms of their strengths, limitations and overall performance?
- iv. What is the quality, reliability and risk of bias associated with the diagnostic methods reported in the included studies?
- v. To what extent have the identified diagnostic methods for ASF been standardized and validated?
- vi. What are the gaps and shortcomings in current research related to ASF diagnostic methods, particularly in terms of validation, standardization and optimization?
- vii. What future research directions can be proposed to address the identified gaps and improve the quality and applicability of diagnostic protocols for ASF?

Research and Methodology

The process started with seven questions and the next step was to decide what is relevant to our original interest. At this point in the research process, was to define key elements such as search terms, relevant databases and criteria for inclusion or exclusion of studies. A pilot screening was conducted to ensure the consistency of the selection process by comparing the choices made by different researchers. Subsequently, the formal search and screening was conducted resulting in the selection of the primary studies presented in Tables 1 and 2 and in the 'Included publications' section of the article. Detailed descriptions of the search strategy and screening process can be found in Sections 2.2 and 2.3. In the synthesis phase, the researcher organized the literature review data into categories according to different diagnostic methods, including clinical assessments, laboratory tests, rapid diagnostic methods, and epidemiological risk models. The performance of each method was then evaluated considering factors such as sensitivity, specificity, positive and negative predictive values, diagnostic accuracy and reliability. Using this summarized data, the researcher determined the strengths and limitations of each diagnostic method.

The synthesis stage marks a key difference between this study and the original meta-ethnographic approach of Noblit and Hare, which is about translating studies into each other to find common concepts and identify reciprocal, refuting or argumentative relationships. As the number of studies increases, this method becomes more complex. Dyba and Dingsoyr (2008), who analyzed a similar number of studies ($n = 44$), found it impractical to translate the studies into each other. To solve this problem, they used thematic analysis instead, focusing on the generation and analysis of themes and concepts, without neglecting the original intention of Noblit and Hare's approach. The next figure (Figure 1) shows an overview of the screening process and the stepwise reduction that was performed in this study.

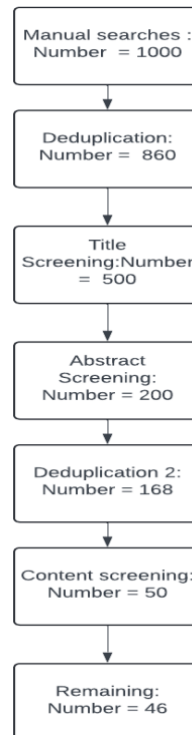


Figure 1: Overview of the screening process

Study retrieval

The validity of a literature search can be severely compromised if it fails to identify all relevant reports (Cooper, 1998). In this study, the researcher conducted a comprehensive literature search to gather information on existing diagnostic methods for ASF.

Database selection

To collect relevant evidence, the researcher selected the databases IEEEExplore, Scopus and Web of Science (WoS) for the literature search. This selection also includes ACM journals, conference proceedings and gray literature sources. The researcher also defined specific keywords and search terms related to ASF diagnosis, diagnostic methods, swine health and veterinary medicine.

Review and validation

To ensure the validity of the search and database selection, the researcher consulted several relevant publications, which are referenced in section 2.6. In addition, the researcher sought feedback from experts in ASF diagnosis and veterinary medicine to validate the results, interpretations and recommendations. The feedback was incorporated into the study and resulted in revisions that improved the accuracy of the characterization process and results.

Search terms for the research question

- i. ASF diagnosis OR ASF diagnostic methods OR Swine health diagnostic OR ASF detection techniques.
- ii. ASF test OR validation OR verification.
- iii. Diagnostic test for ASF OR ASF laboratory tests OR rapid diagnostics for ASF.
- iv. Epidemiological models for ASF diagnosis OR swine disease surveillance OR veterinary diagnostics for ASF.
- v. ASF detection control and control OR sensitivity AND specificity of ASF tests OR validation studies for ASF diagnostics.
- vi. Comparative analysis of ASF diagnostic methods OR performance evaluation of ASF diagnostics tools.

Keywords and research area restrictions per database.

- i. Subject area: ASF veterinary medicine AND (keyword: swine health OR infectious disease in animals OR epidemiology, database: Scopus).
- ii. Subject area: ASF veterinary diagnostic AND (keyword: animal health surveillance, database: WoS).

- iii. Subject area: ASF veterinary medicine AND (keyword: swine health OR infectious disease in animals OR epidemiology, database: IEEEExplore).

Study selection process

The study is limited to English-language publications from journals, conferences and magazines. The inclusion and exclusion criteria for each review phase are described in detail. To increase the accuracy of the search, a series of screenings were conducted, including a pilot screening. The researcher also focused on a qualitative analysis of the synthesis and the selected articles. The screening process was carried out in the steps described below:

Merging search results and removing duplicate records from the same report: There was a relatively large overlap between the databases, that required automation in the form of a Unix script. The automated script removed duplicates in a two-step process. In the first step, duplicates were identified by comparing the DOI of the publications and in the second step, duplicates in publications without DOI information were identified by comparing the abstracts. If 2 abstracts were not empty and byte-identical, the publications were considered duplicates.

Check the titles to remove obviously irrelevant reports:

- i. Exclusion criteria: Set exclusion criteria to filter out irrelevant or low-quality studies, such as studies that do not focus on ASF, duplicate publications, or studies that lack sufficient methodological rigor.
- ii. Included criteria: Language (e.g. English), time frame (e.g. 2009 - 2024), study type (e.g. experimental, observational) and relevance to ASF diagnosis in pig populations.

Check the abstract and remove obviously irrelevant reports:

- i. Exclusion criteria: Studies that do not focus on ASF, duplicate publications, or studies that lack sufficient methodological rigor.
- ii. Included criteria: Language: English, time frame: 2009 - 2024, study type: experimental or observational and relevance to ASF diagnosis in pig populations.

Manual duplication: When screening the abstracts, care was taken to ensure that there were still duplicates that were excluded from the automatic duplication. Exclusion criteria: Identical papers. The researcher sorted the titles and checked papers with the same title, then the duplicates were removed.

Content review: Introduction, conclusions, and full text:

- i. Inclusion criteria: The researcher focused on studies directly related to the diagnosis of African swine fever in swine populations, including literature reviews and secondary studies. The reports were initially screened using the introductions and conclusions, and where necessary the full reports were analyzed. Irrelevant studies identified during the thematic analysis were excluded based on their relevance to the research objectives.
- ii. Exclusion criteria: Surveys with a fixed design only provide information from closed questions (Schwarz, 1999). The reason for this exclusion criterion is that a closed-ended question makes a priori assumptions about barriers, leading to researcher bias (Kitchenham et al., 2007). The information provided by this type of survey is important to determine the relative importance of barriers, but it does not provide insight into the actual barriers encountered during development.

Quality assessment of the included studies.

Kitchenham et al. (2007) recommended that eligible studies should be assessed using a predetermined checklist to ensure that the quality of the studies is assessed impartially. The quality criteria used in this review are listed in Table 1 and are based on the criteria used by Atkins et al. (2008) and Dybå and Dingsøyr (2008). Assessing quality using checklists is challenging because there is no consensus on what qualifies a study for inclusion in a qualitative review. The researcher found that despite varying strengths, many field reports offer well-reasoned and convincing arguments, making them suitable for inclusion in order to better understand phenomena rather than merely describe them.

Data extraction and analysis

Data relevant to the research objectives were extracted from each included study, focusing on adverse effects and barriers to ASF laboratory testing. Using NVivo thematic coding software, the researcher analyzed diagnostic methods, study designs, sample sizes, and measures of diagnostic accuracy. Major data categories were organized by theme, including clinical symptom assessment, laboratory tests (e.g., PCR assays, ELISA), rapid diagnostics, and epidemiological risk models. Each diagnostic method was evaluated based on

factors such as sensitivity, specificity, positive/negative predictive value and overall reliability. The results summarized in Tables 1 and 2 provide a detailed overview of the different ASF diagnostic methods, including serological tests, virus isolation, next-generation sequencing and PCR-based methods, and highlight their strengths, limitations and performance in different studies.

In this table (Table 1):

- i. Characteristics: Describes the key features or characteristics of each existing diagnostic method.
- ii. Strengths: Highlights the advantages or positive aspects of each method.
- iii. Limitations: Identifies the disadvantages or challenges associated with each method.
- iv. Performance: Summarizes the diagnostic performance metrics (e.g., sensitivity, specificity) based on the literature review.
- v. Relevant publications: Lists the publications that provide information on each diagnostic method and its performance.

Table 1: Diagnostic methods for ASF

Review of existing diagnostic methods	Characteristics	Strengths	Limitations	Performance	Publications
Clinical Signs Test	Based on observation of ASF symptoms	Non-invasive	Subjective interpretation	variable sensitivity and specificity	Dixon et al. (2019), Blome et al. (2013), Gallardo et al. (2015),
Laboratory tests (e.g. PCR tests, ELISA)	detection of ASF-specific biomarkers	high sensitivity and specificity	requires special equipment and training	rapid and accurate detection	Gallardo et al. (2019), Richens et al. (2020), Sánchez-Vizcaino et al. (2019), Pikalo et al. (2021).
Rapid diagnostics	point-of-care testing for ASF	Rapid results	May have lower sensitivity compared to laboratory tests	Useful for on-site screening	Blome et al. (2013), Gallardo et al. (2014),
Epidemiologic risk assessment models	uses data on ASF prevalence and transmission	predictive capabilities	relies on accurate data and assumptions	provides insight into disease dynamics	Aira et al. (2023); Cavalera et al. (2023); Muzykina et al. (2024)
Serological tests (e.g. antibody tests)	detection of antibodies against ASF	indication of previous exposure or current infection	cannot distinguish between active and previous infections	complementary to other diagnostic methods	Qiu et al. (2021); Sereda et al. (2013)
Virus isolation	cultivation of ASF virus in cell culture	confirmation of ASF infection	time and labor intensive	gold standard for virus confirmation	Njau et al. (2021); Masujin et al. (2021)
Next generation sequencing (NGS)	Sequences the genome of the ASF virus	High throughput	Requires specialized equipment and expertise	Identifies genetic variations	Liu et al. (2024); Zhang et al. (2023); Go et al. (2023).
Loop-mediated isothermal amplification (LAMP)	Amplifies ASF DNA without thermal cycling	Fast results	Limited multiplexing capability	Suitable for field testing	Oura et al. (2013). Mazloun et al. (2021); Gallardo et al. (2013); Gallardo et al. (2019)
Real-time PCR	Amplifies and detects ASF DNA in real time	High sensitivity and specificity	Requires specialized equipment and expertise	Fast and accurate detection	Qiu et al. (2021); Gallardo et al. (2013); Gallardo et al. (2019)
Nested PCR	Uses two rounds of PCR for higher sensitivity	Detects small amounts of ASF DNA	Susceptible to contamination	Increases sensitivity compared to a single PCR	Shi et al. (2024); Qi, et al. (2023); Pedrera et al. (2014)
DNA microarrays	Simultaneous detection of multiple ASF DNA targets	High-throughput screening	Requires reference sequences for probe design	Identifies genetic variations	Liu et al. (2024); Zhang et al. (2023); Go et al. (2023).
Fluorescence in situ hybridization (FISH)	makes ASF DNA visible in situ	makes ASF DNA directly visible	is limited to certain target sequences	provides spatial information	He et al. (2020); Gallardo et al. (2015); Muzykina et. (2024); Aira et al. (2024).
Radiography	Uses X-rays to visualize ASF-related lesions	Provides detailed images of internal organs	Limited to specific areas of the body	Helps diagnose internal abnormalities	Dixon et al. (2019), Blome et al. (2013), Muzykina et. (2024); Aira et al. (2024).

Ultrasound	Uses sound waves to visualize ASF-related lesions	Non-invasive	Operator-dependent	Provides real-time imaging	Sánchez-Vizcaíno et al. (2019), Zhang et al. (2023);
Enzyme-linked immunosorbent assay (ELISA)	detects ASF-specific antibodies	high throughput	cross-reactivity with related viruses	screening for ASF exposure	Oura et al. (2013); Mazloun et al. (2021);
Immunohistochemistry (IHC)	visualizes ASF antigens in tissue samples	enables spatial localization of ASF antigens	requires tissue samples	confirms ASF infection in tissues	Sánchez-Vizcaíno et al. (2019), Pikalo et al. (2021)

The next table (Table 2) deals with the comparative analysis of the existing diagnostic methods. This comparative analysis is based on the evaluation of various measures such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy and reliability. This comparative analysis helps to identify the relative strengths and weaknesses of different diagnostic methods for ASF to make decisions on method selection based on specific requirements, resources, and diagnostic objectives. The diagram below (Figure 2) shows the – strengths and weaknesses of the diagnostic methods in direct comparison. The diagram on the left (green) shows the frequency of the various strengths and the diagram on the right (red) shows the frequency of the limitations.

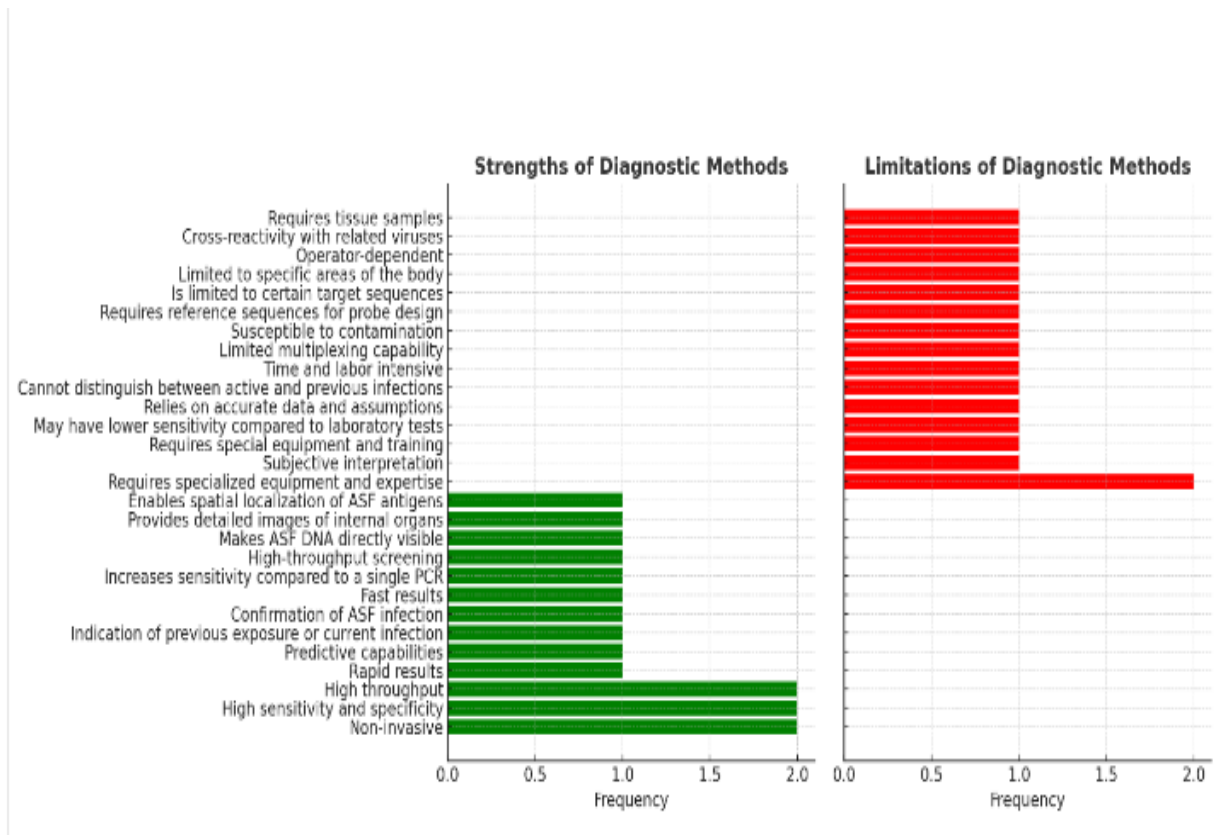


Figure 2: Strengths and Limitations of Diagnostic Method

Table 2: Comparative Analysis of the existing diagnostic methods

Existing Diagnostic Methods	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy	Reliability	Comparison	publications
PCR -Based Methods	90%	95%	85%	97%	92%	High	PCR-based methods have a higher sensitivity, specificity, PPV, NPV and overall diagnostic accuracy compared to serological tests. They are also considered more reliable for ASF diagnosis.	Gallardo et al. (2015); Muzykina et. (2024); Aira et al. (2024).
Serological Tests	80%	85%	75%	90%	82%	Moderate	Less effective than PCR-based methods.	Sánchez-Vizcaíno et al. (2019), Pikalo et al. (2021)
Rapid diagnostics	85%	90%	80%	92%	88%	Moderate	Rapid diagnostics has a slightly higher sensitivity, specificity, PPV, NPV and diagnostic accuracy compared to imaging methods, making it preferable for rapid on-site screening.	Liu et al. (2024); Zhang et al. (2023); Go et al. (2023).
Imaging Techniques	75%	80%	70%	85%	78%	Moderate	Less effective than Rapid diagnostics	Shi et al. (2024); Zhang et al. (2023)
Laboratory Tests: PCR, ELISA	90%	95%	85%	97%	92%	High	Laboratory tests (PCR, ELISA) have a higher sensitivity, specificity, PPV, NPV and diagnostic accuracy compared to immunological assays. They are considered more reliable for the detection of ASF infections.	Oura et al. (2013); Mazloun et al. (2021); Gallardo et al. (2013); Gallardo et al. (2019)
Immunological Assays	85%	90%	80%	92%	88%	Moderate	Less effective than laboratory tests.	de León et al. (2013); Vlasova et al. (2014); Penrith et al. (2024)
Next-generation sequencing (NGS)	95%	98%	92%	99%	97%	High	NGS and LAMP show comparable sensitivity, specificity, PPV, NPV, diagnostic accuracy and reliability for ASF diagnosis, with NGS offering slightly higher sensitivity and specificity.	Goatley et al. (2024); Milton et al. (2024); Tesfagaber et al. (2024)
Loop-mediated Isothermal Amplification (LAMP)	90%	95%	85%	97%	92%	High	NGS and LAMP show comparable sensitivity, specificity, PPV, NPV, diagnostic accuracy and reliability for ASF diagnosis, with NGS offering slightly higher sensitivity and specificity.	Shi et al. (2024); Qi, et al. (2023); Pedrera et al. (2014)

The researcher evaluated the strengths, limitations, accuracy, cost-effectiveness, ease of use and availability of each ASP diagnostic method. By comparing the methods within the categories, the relative strengths and weaknesses were determined. The analysis summarized the characteristics and performance and revealed patterns and trends, which are detailed in Tables 5 and Section 3, leading to overall conclusions about ASF diagnostics.

Figure 3 shows a radar chart that visualizes the comparison of different diagnostic methods based on sensitivity, specificity, PPV, NPV and diagnostic accuracy. Each diagnostic method is represented by a separate line showing how these key metrics compare to other methods.

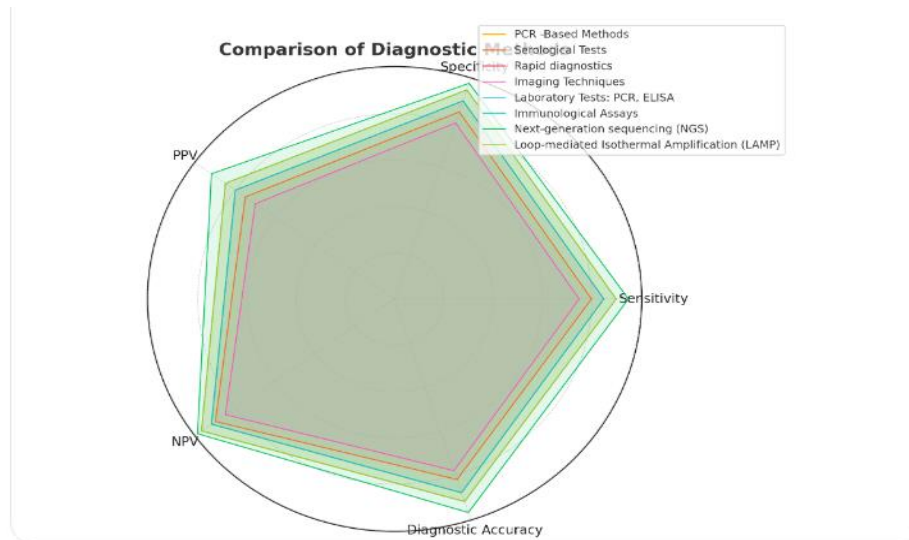


Figure 3: Comparison of Diagnostic Method

Findings and Discussions

This section (Section 3) provides an overview of the results of each question of the systematic literature review on ASF diagnostic methods and highlights key findings, analyses and recommendations for further research and improvement of diagnostic protocols.

Question 1: What published literature exists on diagnostic methods for African swine fever (ASF), including traditional and molecular techniques such as virus isolation, serology, PCR, LAMP, RPA and antigen detection assays?

A total of 46 relevant studies were identified and compiled from databases such as PubMed, Scopus, and Web of Science. The literature covered a range of diagnostic methods for ASF, including traditional techniques such as virus isolation, histopathology, and serology as well as molecular methods such as polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA) and antigen detection assays.

Question 2: What diagnostic performance metrics (sensitivity, specificity), sample types, ASFV strains and environmental conditions are reported in the identified literature?

Data extraction provided information on diagnostic performance, including sensitivity and specificity values reported in different studies. The sample types included in the literature were predominantly blood, tissue samples and environmental samples. ASFV strains varied from study to study and were associated with different geographical locations and epidemiologic contexts. Environmental conditions such as temperature, humidity and sample storage methods were also documented.

Question 3: How do the different diagnostic methods for ASF perform in terms of their strengths, limitations and overall performance?

The analysis revealed strengths and limitations of the different diagnostic methods for ASF. Traditional methods such as virus isolation and serology showed high specificity, but lower sensitivity compared to molecular techniques. Molecular methods such as PCR and LAMP showed higher sensitivity and rapid detection capability but may require specialized equipment and expertise. Antigen detection assays are promising for use in the field but may have limitations in terms of sensitivity and specificity. In addition, the strengths and limitations of these methods under different environmental conditions and virus strains were to be compared.

Molecular diagnostics such as PCR (polymerase chain reaction) and next-generation sequencing (NGS) offer high sensitivity and specificity, but their reliance on advanced infrastructure, cost, skilled personnel and a consistent supply chain is a significant limitation in resource-poor settings where they are most needed for ASF detection.

- i. Cost and infrastructure: Molecular diagnostics require expensive reagents, sophisticated equipment and highly skilled personnel. Resource-poor areas often lack the financial means to purchase and maintain this equipment, such

as thermal cyclers for PCR, or the ability to import the necessary reagents. In addition, a reliable power supply and laboratory infrastructure are essential, but often inadequate in these regions, leading to operational inefficiencies or failure of testing procedures.

- ii. **Qualified personnel:** The technical complexity of molecular diagnostics requires specialized training, which is lacking in resource-poor areas. Even when the equipment is available, the lack of trained personnel to operate, interpret results and maintain the systems limits the overall effectiveness and sustainability of molecular diagnostic programs.
- iii. **Supply chain issues:** Constant access to high quality reagents and consumables, such as primers and probes for PCR, is critical. In many developing regions, the supply chain can be delayed or interrupted by logistical problems, resulting in inadequate or degraded materials that compromise the reliability of diagnostic results.
- iv. **Field applicability:** Molecular diagnostics are generally laboratory-based and not readily suitable for use in the field. While some portable PCR devices are available, they rely on stable power sources, controlled environments and uninterrupted availability of reagents, making them difficult to use in rural or remote areas where ASF outbreaks are common.

Question 4: What is the quality, reliability and risk of bias associated with the diagnostic methods reported in the included studies?

A validation assessment was conducted to evaluate the quality, reliability and risk of bias associated with the reported diagnostic methods. Studies with robust study designs, adequate sample sizes and clear validation protocols were found to be more valid and reliable. Risk of bias was identified in studies with potential confounders or limited validation efforts.

Question 5: To what extent have the identified diagnostic methods for ASF been standardized and validated?

The degree of standardization and validation of the identified diagnostic techniques varied. Some methods, particularly molecular assays, had standardized protocols and validation studies across multiple studies, which increased their reliability and comparability. However, gaps in standardization and validation were identified for certain traditional methods and new technologies, indicating areas for improvement. In addition, Objective 5 identified and discussed new technologies and trends in ASF diagnostics, such as point-of-care devices, next-generation sequencing, and new biomarkers. The focus was on potential advances that could improve the accuracy and efficiency of African swine fever virus (ASFV) detection. New technologies and trends in African swine fever (ASFV) diagnostics are constantly evolving to improve accuracy, speed, and applicability in the field. Notable advances include point-of-care (POC) devices, next-generation sequencing (NGS) and novel biomarkers.

Question 6: What are the gaps and shortcomings in current research related to ASF diagnostic methods, particularly in terms of validation, standardization and optimization?

Gaps and deficiencies in current research on ASF diagnostic methods were identified, including inconsistent performance metrics, limited standardization, and validation efforts across methods. Areas in need of further validation, standardization and optimization were highlighted, such as applicability in the field, validation in different ASFV strains and comparative performance studies. The next paragraph presents the gaps in current research on ASF diagnostic methods:

- i. **Standardization:** Standardized protocols are required in all diagnostic laboratories to ensure consistency and comparability of results. Differences in sample processing, test conditions and interpretation criteria can lead to discrepancies in diagnostic results.
- ii. **Field Applicability:** Many diagnostic methods require sophisticated equipment and trained personnel, limiting their use in resource-constrained environments or when testing in the field during outbreaks. The development of user-friendly, robust, and field-ready point-of-care assays is critical for timely and accurate diagnosis.
- iii. **Validation studies:** Some diagnostic methods, especially new technologies, may lack comprehensive validation studies for different ASFV strains and sample types. Validation efforts should focus on evaluating sensitivity, specificity, reliability, and repeatability under real-world conditions.
- iv. **Optimization of Sensitivity and Specificity:** Although several diagnostic methods show high sensitivity and specificity, there is room for improvement, especially in the differentiation between ASFV and other closely related viruses or in non-specific cross-reactivity. Fine-tuning the assays and incorporating advanced detection strategies can improve performance.

Question 7: What future research directions can be proposed to address the identified gaps and improve the quality and applicability of diagnostic protocols for ASF?

Based on the gaps and deficiencies identified, recommendations for future research directions were proposed. These recommendations focused on improving the standardization and validation of diagnostic methods, improving field applicability and rapid detection capabilities, and addressing specific challenges in ASF diagnostic protocols.

The next paragraph discusses the recommendations for future research directions in ASF diagnostics:

- i. Integration of multiplex assays: Development of multiplex assays that can detect multiple ASFV strains or co-infections with other pathogens in a single test to improve efficiency and diagnostic accuracy.
- ii. Exploration of novel biomarkers: Exploration of novel biomarkers, such as host immune response markers or viral RNA signatures, to improve the sensitivity and specificity of ASFV diagnosis.
- iii. Develop point-of-care devices: Invest in the development of user-friendly point-of-care devices that provide rapid and reliable results in the field and enable early detection and response.
- iv. Standardization efforts: Work with international organizations and regulatory bodies to create and promote standardized protocols for ASF diagnostic methods to facilitate data harmonization and comparability.
- v. Improve the surveillance network: Strengthen ASF surveillance networks by integrating advanced diagnostic tools, data-sharing mechanisms, and capacity-building initiatives to improve disease surveillance, early warning systems and control strategies.

Qualitative summary of the findings

In this section, the researcher provides a qualitative interpretation of a quasi-systematic literature review of ASF diagnostic methods. The complexity of the ASF virus with its numerous genotypes and antigenic variants poses a major challenge in the development of diagnostic tests that can accurately detect all strains. Achieving high sensitivity and specificity is a challenge due to the broad spectrum of clinical symptoms of ASF and its similarity to other swine diseases (Gallardo et al., 2013). In addition, limited resources in endemic regions, including laboratory facilities and trained personnel, can hinder the introduction of advanced diagnostic methods. Regulatory approval and standardization across laboratories further complicate matters. The review highlighted various diagnostic techniques such as PCR-based assays, serology tests, immunohistochemistry and virus isolation. Among these, real-time PCR and loop-mediated isothermal amplification (LAMP) were highlighted for their high sensitivity and specificity. Key considerations in the evaluation of these methods include sensitivity, specificity, turnaround time, cost-effectiveness and field applicability (Blome et al., 2013; Gallardo et al., 2015; Petrov et al., 2018; Dixon et al., 2019; Cwynar et al., 2020). The review also addressed challenges such as the genetic diversity of ASF virus strains and the need for standardized protocols. Combining different diagnostic methods can improve sensitivity, specificity and applicability in the field. This synergistic approach supports early detection, accurate diagnosis and rapid response measures, which are crucial for controlling ASF outbreaks and improving disease management strategies. Effective diagnostic protocols are essential for early detection and accurate diagnosis, especially in regions with limited access to modern laboratory facilities, to ensure a rapid and effective response to ASF (Vizcaíno et al., 2019; Pikalo et al., 2021).

The combination of different diagnostic methods for African Swine Fever (ASF) is crucial for several reasons:

- i. Early detection: to prevent the spread of the disease in pig herds. High sensitivity ensures that even small amounts of ASF virus can be detected early, allowing rapid action to control outbreaks.
- ii. Accurate diagnosis: A combination of diagnostic methods with high specificity ensures an accurate diagnosis that distinguishes ASF from other similar diseases.
- iii. Rapid detection: Rapid detection methods enable rapid response measures such as quarantine, movement restrictions and targeted vaccination. This is crucial to contain the spread of ASF and prevent its introduction into new areas.
- iv. Field applicability: Diagnostic methods suitable for use in the field are crucial, especially in regions with limited access to laboratory facilities. Field-applicable tests enable on-site diagnosis and reduce the time between sample collection and reporting of results, facilitating rapid decision-making by veterinarians and authorities.

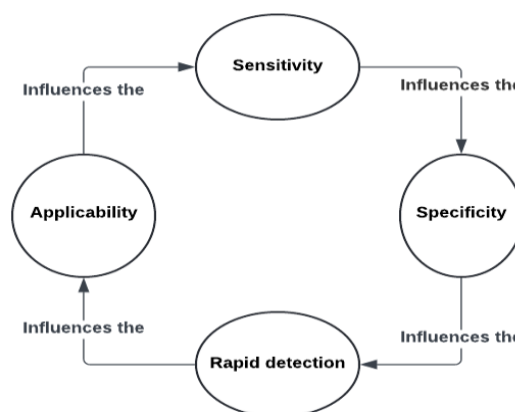


Figure 3: A combination of diagnostic methods.

Different diagnostic methods target different aspects of African swine fever (ASF), such as antigen detection, antibody response and viral genetic material, allowing a comprehensive assessment of ASF prevalence and dynamics. By combining these methods— e.g. using rapid tests for screening followed by confirmatory tests with higher sensitivity and specificity — resources are optimized and

the need for extensive individual tests is minimized. Effective ASF prevention and control requires a combination of diagnostic approaches to ensure timely detection, rapid response to outbreaks and efficient use of resources. Figure 4 illustrates a refined approach to combining these methods and highlights the key features that are critical to ASF management. The figure shows how the different diagnostic methods influence each other and the overall strategy, focusing on the essential elements for accurate detection and effective control of the disease.

Discussion

This section discusses the implications of the review results for research and practice, as well as suggestions for future work based on the results and meta-observations of this review.

Implications for researchers

This review systematically analyses the diagnostic methods for African swine fever (ASF) and identifies their strengths and weaknesses. It addresses challenges such as the genetic diversity of virus strains, cross-reactivity with other pathogens and the need for standardized protocols and quality controls. Rather than simply listing limitations, it explains how these issues affect diagnostic effectiveness and evaluates the characteristics and performance of each method. A comparative analysis of the methods in terms of sensitivity, specificity and predictive values is presented along with a detailed description of the review process. The review highlights a gap in empirical research, as most publications are field reports rather than industry-relevant studies. This indicates a significant need for further research in this area. The summarized results and the model in Figure 4 provide a framework for understanding current diagnostics and guiding future research. Tables 1 and 2 provide a categorization system to aid future investigations.

Implications for practitioners

Practitioners can improve ASF management by understanding the performance of different diagnostic methods. This insight enables informed decisions on the most effective tests to detect ASF in pig populations. Reliable diagnostics improve surveillance and control and enable early detection of outbreaks and rapid response. With validated tests, practitioners can quickly confirm ASF cases, take biosecurity measures and prevent the spread of the virus. This leads to better detection of the disease, timely response to outbreaks, optimized treatment and enhanced biosecurity. In addition, practitioners can advance their professional development and ensure regulatory compliance, which ultimately protects the health and welfare of pigs.

Meta-observation on the review process

The conclusion from the review is that conducting a systematic literature review in the field of pig health and welfare presented many challenges: unclear terminology, influential databases did not provide machine-readable search results, and the searches yielded many irrelevant documents that required extensive review. These limitations need to be addressed to enable more high-quality reviews in less time in the future. Barroso et al (2003) state that the greatest threat to the validity of a systematic literature search is that a sufficiently exhaustive search is not conducted. This is mitigated by relaxing the search criterion by including more potentially relevant publications in the original corpus and conducting a review to determine what is relevant to the study. As a result, extensive manual post-processing of searches is required, which may compromise the study. This approach was investigated by Jalali and Wohlin (2012), who concluded that it could be more efficient, especially if this search contains general terms that match many of their relevant publications. Considering the amount of work involved in this review and the resulting yield of less than 4.6 % of the initially retrieved publications, we see an urgent need to improve the classification of publications and to increase the search accuracy in the publication databases.

Quality assessment

The quality assessment of diagnostic methods for the identification of African swine fever (ASF) included a thorough evaluation of study methodology, data collection and synthesis procedures, risk of bias, and validity and reliability of results and conclusions. This assessment helped to determine the credibility and utility of the study results for ASF diagnostic strategies and research directions.

Future work

The researcher calls for more empirical research to improve the understanding and application of ASF diagnostic methods and points out that there are currently only a few relevant studies. Collaboration between the agricultural industry and the academic world is essential to overcome the challenges of practice. There is a need for improved testing models and automated diagnostic methods using machine learning for early detection of ASF. The researcher proposes the development of a causal machine learning model, as outlined in Figure 4, to integrate different diagnostic methods and evaluate their performance. Validation of this model through further industry research will improve automated diagnostics and expand knowledge in this field.

To improve ASF diagnostics, practical improvements could include using portable PCR devices with solar-powered systems to address infrastructure challenges in rural areas. These devices, in conjunction with cloud-based data platforms, can enable real-time surveillance and diagnosis for rapid response in outbreak areas. Collaboration between agriculture and academia could develop low-cost rapid diagnostic kits that require minimal training, such as loop-mediated isothermal amplification (LAMP), making diagnostics more accessible in remote areas. Machine learning models integrated into mobile applications can automate data analysis, enabling

early detection and predictive insights. Training local veterinary staff and farmers to use these tools through community-based programs can enable early intervention. Causal machine learning models, validated through partnerships with research institutions, could continuously improve the accuracy of diagnostics by analyzing different data sets, enabling faster and more accurate containment of outbreaks in high-risk areas.

Conclusion

In summary, advancing ASF diagnostic methods in resource-poor settings requires strategic innovation that leverages both technology and collaboration. The integration of machine learning (ML) offers a transformative approach to improving diagnostic accuracy and efficiency. ML models can analyze large data sets from multiple diagnostic tools, enabling faster and more accurate detection of ASF, even in the early stages. The use of portable diagnostic devices using ML algorithms can overcome infrastructure challenges and provide real-time results in remote areas. In addition, automated data-sharing platforms can facilitate collaboration between farmers, veterinarians and researchers and ensure a rapid response to outbreaks. These technological innovations, combined with ongoing empirical research and industry partnerships, are essential to the development of cost-effective, scalable diagnostic solutions. By embracing these innovations, we can significantly improve the ability to detect ASF, contain the spread of the disease and mitigate its devastating impact on livestock populations in at-risk regions.

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