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Advancements in Molecular Detection Technology of Senecavirus A: A Comprehensive Review

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Abstract:

In recent years, the *Seneca Valley virus* (SVV) has impeded the sustainable development of the swine industry, posing a major challenge to disease prevention and control in swine populations. The emergence of *Seneca Valley virus* (SVV) presents twofold challenges for swine production systems: it not only significantly interferes with routine farm management protocols, but also substantially complicates clinical differentiation due to its pathognomonic similarity to foot-and-mouth disease (FMD) and swine vesicular disease (SVD). To effectively control the spread of the virus, developing a more convenient and user-friendly rapid detection scheme has become the key focus of disease diagnosis innovation. This paper collected reports on the innovation and application of molecular detection technology of the *Seneca virus*, and sorted out these methods, to provide some scientific basis for the prevention and control of the SVV epidemic in the future, reduce economic losses, and prevent further spread of the virus.

Keywords:

Seneca virus A
Molecular detection
Rapid detection
RPA

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1. Introduction

Seneca Valley virus (SVV), also known as *Seneca Virus A* (SVA), is classified as the sole species within the genus *Senecavirus* (family *Picornaviridae*) [1]. The clinical manifestations of SVV infection in swine populations closely resemble those induced by vesicular diseases, particularly *Foot-and-mouth disease virus* (FMDV) and *Swine vesicular disease virus* (SVDV), as evidenced by comparative histopathological analyses. SVV can cause vesicular lesions on the mouth, nose, and hoof crown of adult pigs, with occasional symptoms such as fever and diarrhea, and increase the mortality of newborn piglets. This makes clinical diagnosis difficult. Also, there is currently no commercially available SVV vaccine. This

has caused a lot of economic losses to the pig breeding industry.

Early diagnosis through validated assays represents a pivotal strategy to control epidemic transmission of SVV in swine populations. The timeliness and accuracy of detection are key determinants for interrupting viral spread, especially within intensive production systems where rapid pathogen transmission occurs via direct contact or fomites. Molecular diagnosis has quickly become a popular pathogen detection method because of its fast detection speed and high sensitivity. Recent advancements in diagnostic technologies have substantially enhanced assay performance, with notable improvements in processing efficiency and analytical sensitivity across multiple detection platforms. In this paper, the molecular detection methods of SVV reported in recent years are sorted out, which provides more choices for the detection methods of this pathogen and also provides a reference for the development of rapid detection technology.

2. Introduction of SVV

Seneca Valley virus (SVV) was incidentally isolated in 2002 from the PER.C6 (transformed fetal retinoblast) cell line [2]. In 2015, Brazil first reported an outbreak of SVV in pigs [3]. Subsequently, the United States [4], Thailand [5], Vietnam [6], Colombia [7], and other countries reported that SVV broke out in their country.

The susceptible animals of *Seneca virus* are pigs, and pigs of all ages are susceptible. Viruses can spread through direct and indirect contact with viral pollutants or aerosols. The pathogenicity and fatality rate of the virus are related to the age, breed, and geographical factors of pigs, and generally occur in spring and autumn [8]. Current epidemiological studies have demonstrated a significant association between SVV infection and elevated mortality rates in neonatal piglets during perinatal stages. The mortality rate of adult pigs is extremely low, usually subclinical infection or recessive infection. The mortality rate of piglets is higher than that of adult pigs, and the incidence rate of sows is as high as 70–90% [9].

3. SVV detection methods

3.1. Pathogenic detection

Virus isolation is the most accurate method to identify and diagnose SVV. It has been found that cells that can be used to isolate the *Seneca virus* include PER.C6 [10], NCI-H1299 [11], HEK293T [12], ST [13], PK-15 [14], and so on. While virus isolation-based detection protocols for SVV demonstrate high diagnostic specificity, their technical complexity, requiring specialized biosafety containment facilities (BSL-2+), and prolonged turnaround time (> 48 hours post-sample collection) render these methods suboptimal for field applications requiring expedited diagnostics during outbreak investigations. The clinical similarity between SVV and FMDV infections in swine poses significant diagnostic challenges. Given that FMDV is a zoonotic pathogen, virus isolation protocols for differential diagnosis necessitate stringent biosafety containment measures (BSL-3), particularly during epizootic investigations where misidentification could amplify public health risks.

3.2. Antibody detection

Antibody detection methods are suitable for handling a large number of samples in epidemiological surveillance or mass diagnostic programs [15]. Serodiagnostic approaches currently implemented for SVV surveillance in swine populations encompass indirect enzyme-linked immunosorbent assay (iELISA) [16], competitive ELISA (cELISA), indirect fluorescent antibody (IFA) testing, and virus neutralization test (VNT) [17,18]. Compared with other Antibody detection assays, ELISA is famous for its high sensitivity, specificity, convenience, rapid, and cost-effectiveness. SVV ELISAs have been developed to detect IgG antibodies against non-structural proteins such as 2C, 3C, 3D, L, and 3AB proteins and Virus-like particles (VLPs) [19,20]. However, serological detection needs a period after virus infection to produce a reliable antibody reaction, so it cannot meet the requirements of rapid detection in the early stage of the epidemic.

3.3. Molecular biological detection

Nucleic acid-based detection methodologies in molecular diagnostics primarily involve polymerase chain reaction (PCR) and its advanced derivative, real-time quantitative reverse transcription PCR (qRT-PCR)

[21]. Currently, these techniques serve as the primary standard for diagnosing animal vesicular diseases due to their rapidity, sensitivity, and strong specificity in pathogen identification. Conventional PCR relies on agarose gel electrophoresis for result analysis. While cost-effective, this method involves biohazard risks from nucleic acid dyes and requires both thermal cyclers and horizontal electrophoresis units, limiting its application in settings with unstable power supply. Moreover, PCR detection requires not only a precise thermal cycler but also a horizontal electrophoresis instrument, which is very inconvenient when there are not enough electricity resources. qRT-PCR detection methods have high sensitivity and can detect rare targets, which is a very mature detection method. However, this method requires professional laboratory equipment and operators. Meanwhile, to ensure accurate temperature control, it is necessary to supply power to the fluorescence quantitative PCR instrument continuously. A multiplex real-time RT-PCR assay for detecting and distinguishing FMDV from SVV was also recently developed and evaluated [22]. The method can identify FMDV and SVV at the same time, aiming at improving the efficiency of disease detection.

3.4. Development of molecular detection technology

3.4.1. Molecular detection method based on polymerase chain reaction technology

Insulated isothermal PCR (iiPCR), a fluorescent probe-mediated nucleic acid amplification system under constant temperature conditions [23]. iiPCR technique achieves nucleic acid amplification through sequential thermal cycling across distinct temperature phases (denaturation, annealing, and elongation) within a microfluidic capillary, facilitated by a portable thermal control system. This approach employs an integrated nucleic acid processing system capable of executing automated PCR amplification and result interpretation within approximately one to one and a half hours. Nucleic acid amplification can be completed in 30–40 minutes. Two molecular assays targeting conserved SVV genomic regions were established for viral RNA detection: a reverse transcription PCR (RT-PCR) assay specific to the 5’UTR and a reverse transcription insulated isothermal PCR (RT-iiPCR) method focusing on the 3D gene, with inter-assay consistency reaching 98.4% [24].

Reverse transcription droplet digital PCR (RT-ddPCR) is considered to be an accurate and sensitive technique, showing good sensitivity and specificity in SVV detection. Beyond diagnostic applications, RT-ddPCR facilitates absolute SVV RNA quantification independent of calibration curve construction [25]. However, this method requires high personnel operation, and it still can’t get rid of expensive precision instrument detection, which can’t meet the requirements of on-site rapid detection [26].

3.4.2. Molecular detection method based on constant temperature amplification technique

Constant temperature amplification technology is a kind of molecular biological detection method at constant temperature. This methodology demonstrates operational simplicity, minimal instrumentation requirements, and rapid processing timelines when contrasted with standard PCR protocols. At present, this technology is mainly divided into LAMP [27], RCA [28], RAA [29], and RPA. Through the engineering of sequence-specific primers and enzyme systems, the exponential amplification of target DNA or RNA can be realized at constant temperature.

Loop-mediated isothermal amplification (LAMP) is an efficient DNA amplification method, which can rapidly amplify the target DNA sequence at constant temperature. LAMP technology uses multiple primers and DNA polymerase to realize continuous amplification of target DNA. LAMP technology shows good sensitivity and specificity in SVV detection, and its operation is relatively simple, so it is a potential rapid detection method.

Recombinant enzyme polymerase amplification (RPA) is a new isothermal amplification technology, that can rapidly amplify the target nucleic acid sequence at low temperature (37–42 ℃) [30]. At present, RPA technology can be divided into three categories, including basic RPA, fluorescent RPA, and lateral flow RPA [31]. The fundamental RPA system comprises four core components: recombinase, ssDNA-binding protein, DNA polymerase, and amplification-optimized buffer formulation. After adding templates and primers, it can react quickly at room temperature, and the results can be judged within 30 minutes by gel electrophoresis.

The principle of fluorescent RPA is similar to that of fluorescent quantitative PCR. During the amplification reaction, this RPA can cut the recognition sites of special fluorescent probes through the nucleic acid exonuclease in the reagent and release fluorescent signals, which can be combined with a fluorometer for real-time quantitative detection. In response to the lack of professional detection equipment in some areas, some companies have developed portable fluorescence meters that can even be powered by solar energy. These portable fluorescence meters are small in size and light in weight, and nucleic acid amplification can be observed in real-time on the spot through RPA fluorescence meters [32].

4. Application of isothermal amplification technology in SVV detection

Recently, isothermal amplification technology has been widely used in the rapid detection of SVV [33]. The researchers designed primers and probes for isothermal amplification of SVV-conserved regions, which showed good sensitivity and specificity in detection. Some researchers combined RPA technology with the CRISPR/Cas12a system to develop an accurate and sensitive diagnosis and detection platform for SVV. This methodology demonstrated an analytical sensitivity threshold of 10 copies per reaction [34]. This method integrates RPA reaction and CRISPR/Cas system in a centrifuge tube, which reduces the risk of sample pollution and has great practical application potential in an environment with limited resources.

5. Summary

At present, most molecular detection technologies need harsh laboratory environments and professional detection personnel. Infectious diseases are more likely to spread in areas with difficult conditions or poor sanitation. Therefore, developing more convenient, sensitive, and fast on-site detection methods is the direction of future research. By sorting out the main molecular detection methods at present, RPA has shown its unique advantages in the field of rapid on-site detection because of its characteristics of rapidity, simplicity, and convenience, and has been gradually paid attention to and applied to the detection of zoonotic pathogens. The reaction temperature of RPA is close to human body temperature and its reaction time is short (5–20 min), which makes it more suitable for on-site rapid detection. In recent years, scholars have been trying to combine RPA with LFD, CRISPR/ Cas system, and other diagnostic techniques to develop more sensitive and convenient molecular diagnostic methods. It has been reported that a nucleic acid detection method without RNA is described, which greatly improves the detection speed [35]. Another multienzyme isothermal amplification technique used by some scholars is called multienzyme isothermal rapid amplification (Mira), which uses a recombinant enzyme named *Streptomyces* azure recA (SC-recA) to improve the reaction stability and anti-interference ability [36]. Researchers have engineered a regenerable dual-channel fiber-optic immunosensing platform (DOFIS) for enhanced diagnostic applications. The detection can be completed in 10 min with low cost and simple operation [37]. All the above rapid detection methods can provide some reference for the development of SVV rapid detection.

In addition, using freeze-drying technology, lateral chromatography test strips, and other technical means, developing portable detection reagents or consumables that are easy to carry and use can better meet the needs of grassroots veterinarians for on-site detection. Future research should further improve the detection sensitivity and specificity, and develop portable detection equipment to provide more powerful support for the prevention and control of SVV. In the future, we will continue to pay attention to and explore the research progress in this field, and make greater contributions to the rapid detection of SVV and the prevention and control of diseases.

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