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Establishment of real-time viral pathogen detection method-surface-enhanced Raman scattering system for porcine circovirus type II

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Abstract

The transition towards large-scale intensive pig farming methods has resulted in an increase in cases of complex, mixed infections, and secondary infections. Porcine circovirus (PCV), the causative agent of PCV disease (PCVD), is a small nonenveloped DNA virus containing a single-stranded circular genome. Previously, PCV was even considered nonpathogenic to pigs. However, a novel strain of PCV, designated PCV-2, has been associated with various disease syndromes in pigs over the last 5 years. PCV-2 primarily induces post-weaning multisystemic wasting syndrome, characterized by clinical signs such as debility, dyspnea, palpable lymphadenopathy, diarrhea, and pallor or icterus. PCV-2 is now recognized as an important emerging pathogen. Early diagnosis and disease prevention have become crucial for effective pig farm management. However, clinical diagnosis may be complicated by antibiotic treatment and atypical disease symptoms. To ensure accurate pathology diagnosis, it is essential to integrate robust laboratory diagnostics with traditional methods. Surface-enhanced Raman Scattering (SERS) spectroscopy has emerged as a potentially powerful technique for whole-organism fingerprinting, enabling rapid identification of bacteria. Biosensors utilizing SERS offer promising capabilities for sensitive and quick detection of bacterial pathogens, thereby reducing diagnosis time. In this study, our aim was to characterize and evaluate a SERS-based diagnostic system for detecting and identifying PCV-2 present in pooled swine sera and feces. We compared the spectra of PCV-2 recovered from the specimens to those of pure PCV-2 to determine the PCV-2 molecular fingerprint. Our results demonstrated successful detection, identification, and classification of PCV-2 in swine sera and feces using SERS. SERS provided reproducible molecular spectroscopic signatures suitable for analytical applications. This approach presents a new and potent tool for real-time surveillance of animal viral pathogens in clinical settings.

Keywords: Animal Diseases; Pig; Porcine Circovirus Type II; Raman Spectrum; Real-Time Surveillance

1. Introduction

With the intensification and continuous expansion of the swine farming industry, pig disease issues have become increasingly complex, with a rise in cases of mixed or secondary infections. Therefore, rapid diagnosis, early prevention, and treatment of diseases demonstrate their importance. Furthermore, due to the use of antibiotics and vaccines, symptoms of bacterial or viral diseases in pigs often present atypically, leading to significant economic losses for

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livestock producers. Thus, relying solely on clinical symptoms for rapid diagnosis would be challenging. Strengthening and developing new laboratory diagnostic techniques are currently the primary choice [1-2].

Porcine circovirus (PCV) is a common virus in pigs, often exhibiting no apparent symptoms like an invisible presence. However, it can suddenly cause illness when encountering suitable growth conditions, affecting both breeding and fattening pigs alike. Prevention and control methods involve strengthening hygiene management, maintaining ventilation in livestock farms, appropriate stocking densities, and correct vaccine administration to reduce the occurrence of circovirus [1-2, 5, 33, 54-55].

Porcine circovirus disease (PCVD) is a viral illness affecting pigs that has recently emerged as a significant global concern. This disease manifests in piglets with symptoms including progressive weight loss, enlarged lymph nodes, respiratory abnormalities, diarrhea, pallor, and jaundice. PCVD poses a substantial threat to the swine industry and has been reported across the globe [1-2].

PCV is a single-stranded DNA virus found in the *Circoviridae* family, specifically in the *Circovirus* genus. Initially, PCV-1 was considered non-pathogenic, but PCV-2 is widespread in swine populations and is linked to postweaning multisystemic wasting syndrome (PMWS) in pigs. PCV-2 is almost ubiquitous in pig herds globally. PMWS is a complex condition usually requiring concurrent infection with other pathogens like porcine parvovirus or porcine reproductive and respiratory syndrome virus for more severe disease manifestation [33].

Epidemiological evidence from PCV outbreaks in vaccinated herds, combined with molecular evolutionary analysis, highlights the constant evolution of PCV, leading to recurrent outbreaks. This evolutionary trait presents challenges to vaccine-induced immunity, complicating disease prevention efforts. Hence, the development of effective vaccines targeting evolving PCV strains is crucial for controlling PCVD outbreaks and reducing economic losses [1-2].

Presently, research efforts for PCVD vaccines encompass various approaches, including modified live virus (MLV) vaccines, inactivated PCV vaccines, and subunit PCV vaccines, among others. Commercially available PCV-2 vaccines mainly consist of inactivated or subunit formulations. Additionally, a live-attenuated PCV-2 vaccine utilizing a chimeric PCV-1-2 strain has been developed. Given PCVD's widespread prevalence in the global pig industry, vaccination remains pivotal in preventing PCV infection [1-2, 54-55].

Studies underscore PCV-2 as a significant pathogen responsible for postweaning multisystemic wasting syndrome (PMWS) in pigs post-weaning. PCV-2 infection impairs pigs' lymphoid tissues while concurrently affecting both cellular and humoral immune systems. Serum surveys reveal nearly universal presence of PCV-2 antibodies among field pigs. The production and decline of these antibodies, besides reflecting the immune system's response to viral stimulation, may also modulate PCV-2 replication and influence PMWS progression. Notably, the expression of neutralizing antibodies may play a critical role in regulating PCV-2 viral load [33, 54-55].

In recent years, polymerase chain reaction (PCR) has emerged as a widely utilized technique for amplifying targeted DNA sequences with remarkable accuracy and speed. This method facilitates the rapid amplification of specific nucleic acid sequences, greatly enhancing detection capabilities. PCR operates on principles that mimic natural DNA synthesis, including denaturation, annealing, and extension processes.

By designing a pair of primers derived from the PCV-2 gene, it becomes feasible to selectively amplify a precise nucleic acid fragment from a given specimen. This approach can be effectively applied to secretions or organ samples obtained from infected animals. Compared to conventional virus isolation or pathological dissection methods, PCR offers significant advantages in terms of both sensitivity and speed, with testing processes typically completed within a few hours [26].

Raman spectrometer is a device utilized for measuring the spectrum of Raman scattering light. Raman scattering, discovered by Indian scientists in 1928, differs from conventional laser scattering in that its wavelength undergoes slight variations from the original incident light. These variations occur due to collisions between molecules and photons, which either increase or decrease the energy of the photons. This phenomenon is influenced by molecular bonding and structure. As a result of its unique properties, Raman spectrometry finds widespread application in various fields such as polymer detection, nanomaterial analysis, electrochemistry, semiconductor research, thin film characterization, mineralogy, and carbide identification [3-4, 7-9, 11-15].

In recent years, with advancements in charge-coupled device (CCD) and laser technologies, Raman spectrometers have gained increasing importance in rapid biological and pharmaceutical testing. Surface-Enhanced Raman Scattering

(SERS) is now utilized to amplify signals required for specific analyses, particularly in life science research areas such as pharmaceuticals, clinical experiments, cell research, immunology, protein array studies, genetics, genetic engineering, plastic surgery, biomedical materials, environmental engineering, and biosafety [16-20].

In life sciences research, Raman spectrometers offer the remarkable capability to identify and differentiate samples with single-cell precision. The integration of Raman spectroscopy with microscopic imaging techniques holds immense potential for significant advancements in the life sciences industry. Advancements in technology have led to a deeper understanding of SERS. This phenomenon entails the enhancement of Raman scattering signals of adsorbed molecules on specially prepared metal conductors within the excitation region, due to the heightened electromagnetic field on or near the sample surface. SERS has proven invaluable in providing structural information not easily attainable through conventional Raman spectroscopy. Presently, SERS finds extensive application in studying material surfaces, adsorption interface states, configurations of biological small molecules, molecular conformations, and structural analysis. It also aids in analyzing adsorption orientation, changes in adsorption states, and interface information of compounds. Notably, gold, silver, copper metals, and a few alkali metals like lithium and sodium exhibit strong SERS effects, with gold, silver, and copper requiring surface roughening treatment to achieve optimal results. Advances in nano-coating technology and substrate preparation techniques further enhance the SERS signal, contributing to its growing application and attention [23-32].

The peaks detected by Raman spectrometers, termed "characteristic peaks," vary among different biological samples due to differences in composition and structure. They can even discern variations in protein or peptide chains resulting from genetic changes. Thus, differences can be analyzed without molecular biological testing, and changes in the proportion of characteristic peak ratios can aid in tracking differences in RNA or protein expression. Additionally, Raman imaging technology holds promise for drug localization tracking and determining their effectiveness [34-40].

Biological detection is indispensable in the realm of biotechnology, playing a pivotal role in species identification, understanding cellular interactions, analyzing signaling substances, and more. Its significance spans from fundamental research to clinical applications in laboratory testing, drug design, and human disease research. While significant technological advancements have propelled the biotechnology industry forward, each biological technique has its limitations.

For instance, fluorescent labeling, a commonly used technique, can disrupt cellular structures, hindering long-term observation of cell growth changes. The growth time of bacteria directly impacts testing duration, indirectly limiting the speed of bacterial identification. Moreover, inadequate concentration may render signals challenging to detect, necessitating other techniques for substance extraction or signal amplification, thereby indirectly constraining biological detection technology [41-50].

Given these challenges, the integration of biomedicine and optoelectronics has emerged as a key focus in global biotechnology development. Combining these fields is essential for accurate disease diagnosis. With advancements in optoelectronic technology, Raman spectroscopy offers a promising avenue to explore molecular-level mechanisms, functions, and structures within cells. SERS, leveraging the surface plasmon effect, holds immense potential for developing rapid clinical pathogen identification and detection systems. Currently, in the realm of human medicine, SERS has shown success in laboratory settings for detecting bacteria, viruses, and malaria, achieving impressive results with detection limits as low as 1 bacterium and testing completed within 5 minutes. SERS is also utilized clinically for assisting in diagnosis, with urine sample testing from clinical patients achieving detection limits ranging from 1,000 to 10,000 cells and completed within just 3 minutes. Therefore, SERS holds great promise and has the potential to replace traditional detection methods in the future. Furthermore, applying SERS to animal health management can significantly reduce detection time, promptly identify animal infection statuses and prevalent diseases on farms, and aid in controlling disease spread and monitoring animal epidemics.

2. Material and Methods

2.1. Chemicals and Reagents

10% Formalin (Cayman, CAS 50-07-7, Item No.11435), Giemsa Stain Kit (Beso Enterprise, WGO-020), phosphate-buffered saline (PBS; Sigma-Aldrich, Cat. No. P3813), Saline (Shindong Biotech Co., Ltd.), Geneaid Genomic DNA mini kit (Cat. No. GB1000, Labturbo virus mini kit (Cat. No. LVN 480-300), Dream Taq Green PCR Master Mix (2×) (Thermo Scientific, Cat. No. K1081), Taq DNA polymerase (5 U/L, NEB, Cat. No. M0267L or Cat. No. M0267S), PCR reaction buffer (10× Thermopol reaction buffer, NEB, Cat No. B9004S), dNTPs (10 mM, Thermo Scientific, Cat. No. R0192), agarose gel (0.5% syneral gel, Diversified Biotech, Inc., Newton Centre, MA, USA, supplemented with 0.5% agarose, Promega

Corporation, Madison, WI, USA), TBE (8.9 mM Tris, 8.9 mM Borate, 0.2 mM EDTA), 6× loading dye, (Thermo Scientific, Cat. No. SM0323), ethidium bromide (EtBr; 10 mg/mL, Fluka, Cat. No. 46067), DNA marker (100 bp DNA marker, 0.05 mg DNA/mL, Thermo Scientific, Cat. No. SM0323), 99.8% alchol (Merck, Cat. No. 1.00983.2500), primers (Table 1), and PCV-2 (kindly provide from Dr. Chi-Ming Chen) were used in this study.

2.2. Polymerase Chain Reaction Detection

PCR assay for PCV-2 was conducted following the protocols and modification [26]. For the PCR reaction, DNA extract was added to a PCR mixture with final concentrations of 0.5 µL primer-1C478 (5 µM), 0.5 µL primer-2C16R (5 µM), 12.5 µL Dream Taq Green PCR Master Mix (2×), 8.5 µL DDW, and 2.5 µL DNA template per 25 µL. The PCR reaction conditions for PCV-2 was as follows: initial DNA denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 20 seconds, annealing at 62°C for 20 seconds, extension at 72°C for 20 seconds, and final extension and completion of DNA strands at 72°C for 7 minutes, with a final temperature return to 4°C. Finally, the resulting PCR products were subjected to electrophoresis on a 1.5% agarose gel, stained with EtBr, and then photographed and analyzed for test results (Figure 1).

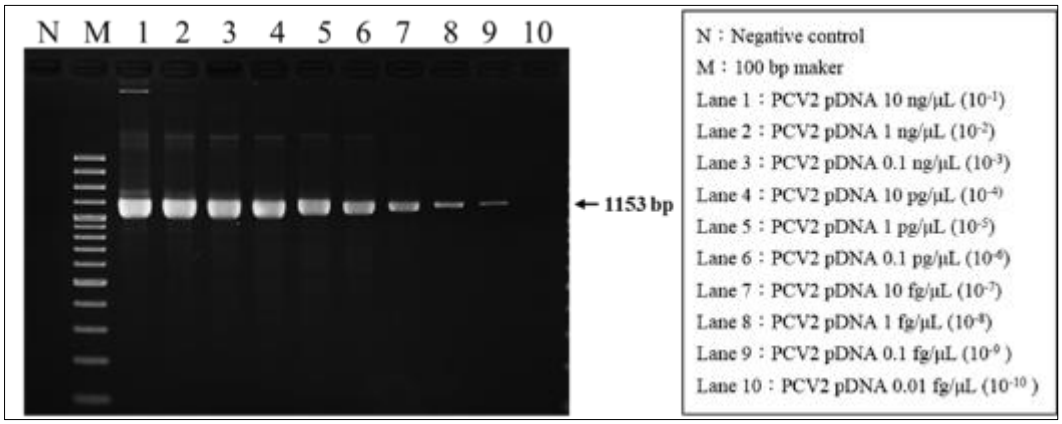

Figure 1 PCV-2 pDNA, negative control will yield PCR products depending on the primer combinations used. The length of the PCR product for PCV-2 is 1,153 bp, while the negative control will not show any PCR product.

Table 1 Primer design of polymerase chain reaction and references of PCV-2

Virus	Sequence (5'→3')	PCR product sizes (bp)	References
PCV-2	1C478：5'-CCG CGG GCT GGC TGA ACT T-3' 2C16R：5'-ACC CCC GCC ACC GCT ACC-3'	1,153	[26]

2.3. SERS Sample Preparation

2.3.1. Swine Fecal Sample Pre-Processing

Pig fecal samples were collected directly aliquot into microcentrifuge tubes, label, and store at -20°C for testing within 2 months. Take 0.015 g of fecal samples and add it to 20 µL of sterile normal saline for resuspension (proportional resuspension). Place it at 4°C for 30 minutes, then centrifuge at 2,000 rpm at 4°C for 15 minutes. Remove the supernatant, then centrifuge at 5,000 rpm at 4°C for 10 minutes and discard the supernatant. Add 500 µL of sterile 0.15 M Sodium chloride solution, centrifuge at 5,000 rpm at 4°C for 10 minutes, and repeat this step twice. Then add 500 µL of sterile deionized water, centrifuge at 5,000 rpm at 4°C for 10 minutes, and repeat this step once. Add 10 µL of sterile deionized water for resuspension (proportional resuspension), and proceed with SERS detection.

2.3.2. Swine Serum Sample Pre-Processing

Pig blood samples without anticoagulants were collected and used a high-capacity centrifuge at 4°C, 3,000 rpm for 15 minutes. The upper clarified liquid is serum, which is then aliquoted into microcentrifuge tubes, labeled, and stored at -20°C for testing within 2 months. Take 150 µL of serum sample, centrifuge at 5,000 rpm at 4°C for 10 minutes, and

discard the supernatant. Add 500 µL of sterile 0.15 M Sodium chloride solution, centrifuge at 5,000 rpm at 4°C for 10 minutes, and repeat this step twice. Add 500 µL of sterile deionized water, centrifuge at 5,000 rpm at 4°C for 10 minutes, and repeat this step once. Add 10 µL of sterile deionized water for resuspension (proportional resuspension), and proceed with SERS detection.

2.4. Raman Spectrometer Detection

The prepared sample suspensions were processed and 2 µL were applied onto the detection chip (Phan^2 SERS). Subsequently, analysis was conducted using RAPID Surface Raman Spectroscopy Rapid Detection Cloud System. Access the webpage https://phanscolab.com/Phansco/SignIn and enter user information. Turn on the main power switch of the SERS system, enter the username and password to log in, and input aliases in the sample description and detection unit sections. In the measurement type section, select substrate validation for chip calibration and confirmation, then press the substrate confirmation to conduct chip substrate measurement until substrate validation is completed. Choose unclassified for the measurement type, drop the tested sample onto the chip, place it into the SERS, press for real-time measurement, and measure the tested sample. After waiting for 10 seconds, the system enters the "cloud comparison" process, and upon completion of cloud comparison, the results will be displayed in the system message column and report. Log in to the cloud system with user ID and password, download the measured Raman spectra from the cloud system, and proceed with spectral interpretation. The machine conditions were set as dimensions: 46 × 35 × 20 cm (including handle), Weight: < 10 kg, Raman light source: 785 nm laser, power: ≤ 100 mW, spectral range: 200-1,800 cm^{-1}, resolution: < 10 cm^{-1}, and operating interface: dedicated detection software compatible with Windows® system. Raman spectrum of PCV-2 was obtained, and the resulting spectrum was analyzed to establish a standard reference database for PCV-2 Raman fingerprinting patterns.

2.5. Statistical Analysis

SERS detection analysis software SpectraView was performed via exporting the SERS data from RAPID Surface Raman Spectroscopy Rapid Detection Cloud System.

3. Results

3.1. Establishment of Raman spectrum of pure PCV-2

PCV-2 was deactivated with 10% formalin and used as the target pathogen for SERS detection. The 2 µL of the virus solution was dropped onto the Raman detection chip for PCV-2 Raman spectrum pattern measurement. Based on previous preliminary test results, the wavelength range from 500 to 1,650 was selected for PCV-2 Raman spectrum pattern comparison. The Raman spectrum pattern of PCV-2 was compared with that of sterile deionized water (ddH_2O) or 10% formalin solution. The results showed 7 significant differences in signal intensity at wave numbers, including characteristic peaks at 523 cm^{-1}, 814 cm^{-1}, 915 cm^{-1}, 1,173 cm^{-1}, 1,292 cm^{-1}, 1,373 cm^{-1}, and 1,580 cm^{-1} (Figure 2, Table 2).

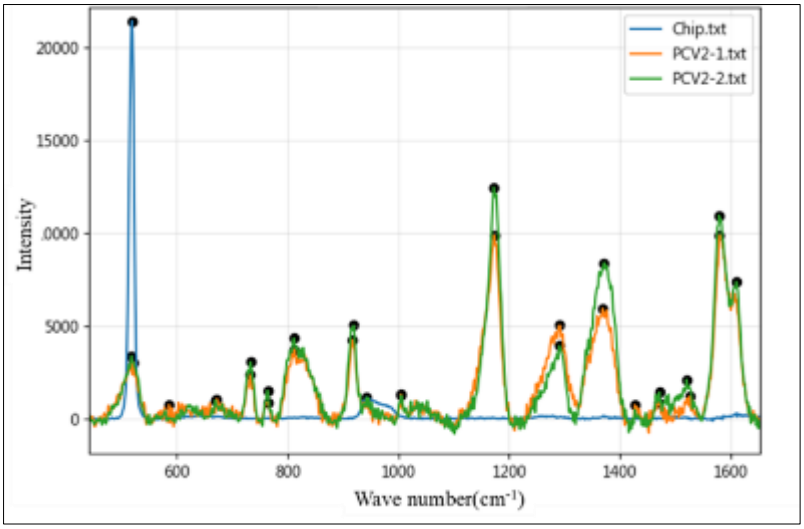

Figure 2 Raman spectra of pure PCV-2. Blue line: background; orange line: pure PCV-2 sample I; green line: pure PCV-2 sample II

Table 2 Wavenumbers (cm^{-1}) of the characteristic Raman peaks of pure PCV-2

Wave number（ cm^{-1} ）
523
814
915
1,173
1,292
1,373
1,580

3.2. SERS detection of pig serum and feces samples mixed with pure PCV-2

Pig serum and feces samples were individually mixed with PCV-2 fixed with 10% formalin, followed by sample pretreatment and SERS detection. The results showed that PCV-2 mixed with samples could be detected with the characteristic Raman peaks. In pig serum samples, 6 significant differences in signal intensity at wave numbers were observed compared to sterile deionized water or 10% formalin solution, including wave numbers 515 cm^{-1}, 806 cm^{-1}, 923 cm^{-1}, 1,180 cm^{-1}, 1,389 cm^{-1}, and 1,615 cm^{-1}. In pig feces samples, 9 significant differences in signal intensity at wave numbers were observed, including wave numbers 523 cm^{-1}, 799 cm^{-1}, 920 cm^{-1}, 1,031 cm^{-1}, 1,172 cm^{-1}, 1,276 cm^{-1}, 1,374 cm^{-1}, 1,475 cm^{-1}, and 1,581 cm^{-1} (Figure 3).

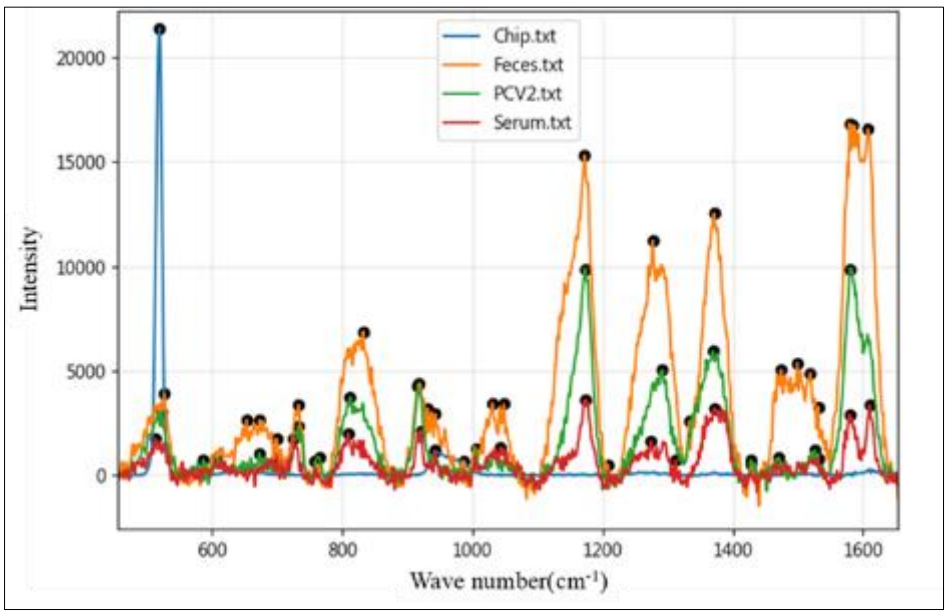

Figure 3 Raman spectra of pig serum and fecal samples respectively were mixed with PCV-2 then performed SERS detection. Blue line: background; green line: PCV-2 sample; red line: pig serum samples were mixed with pure PCV-2; orange line: pig fecal samples were mixed with pure PCV-2

3.3. PCR results of PCV-2

PCV-2 was serially diluted by 10-fold, and SERS detection was performed, followed by comparison with PCR results. The PCR results showed that PCV-2 was not detectable at a dilution of 10^9. Similarly, no amplification products were detected in serum and fecal samples at a dilution of 10^9 (Figure 4A). PCR results of pig serum and feces samples did not been detected PCV-2 (Figure 4B).

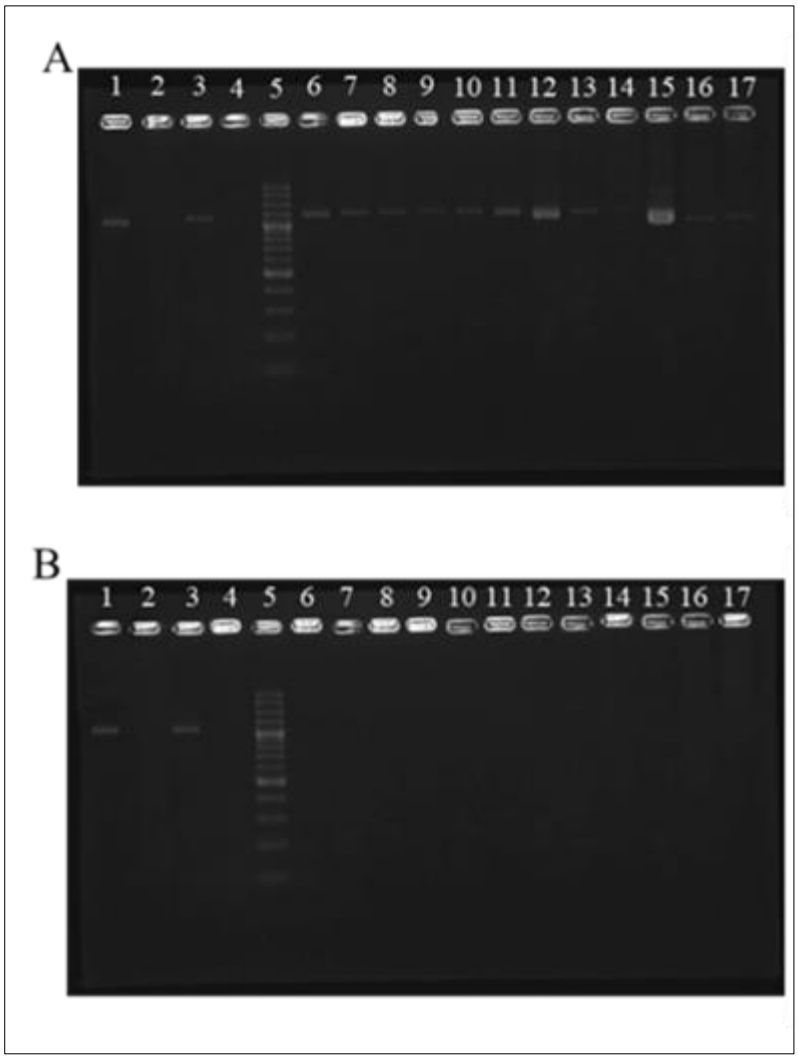

Figure 4 Comparison of PCR and SERS results for PCV-2. (A) PCR results for pure PCV-2 and the mixed pig serum-pure PCV-2 samples and pig feces samples-PCV-2 samples. Lane 1: positive control 10^7× dilution; Lane 2: negative control 10^8× dilution; Lane 3: positive control 10^7× dilution; Lane 4: negative control 10^8× dilution; Lane 5: 100 bp marker; Lane 6: pDNA 10^4× dilution; Lane 7: pDNA 10^5× dilution; Lane 8: pDNA 10^6× dilution; Lane 9: pDNA 10^7× dilution; Lane 10: pDNA 10^8× dilution; Lane 11: pDNA 10^9× dilution; Lane 12: 10^7× dilution of serum; Lane 13: 10^8× dilution of serum; Lane 14: serum 10^9× dilution of serum; Lane 15: feces 10^7× dilution of feces; Lane 16: 10^8× dilution of feces; Lane 17: feces 10^9× dilution of feces. (B) PCR results for pig serum and feces samples. Lane 1: 10^7× dilution of positive control; Lane 2: 10^8× dilution of negative control; Lane 3: 10^7× dilution of positive control; Lane 4: 10^8× dilution of negative control; Lane 5: 100 bp marker; Lane 6-11: 10^8× dilution of serum sample 1-6; Lane 12-17: 10^8× dilution of feces sample 1-6

4. Discussion

In recent years, biophotonics has emerged as a key area of focus in global biotechnology, employing optical technologies to detect biological responses, including the mechanisms, functions, and structures at the molecular level of cells [51-53]. In clinical medicine, it offers non-invasive methods for detecting, diagnosing, and treating diseases in humans. Consequently, it represents an interdisciplinary field integrating biomedical sciences, biotechnology, optics, electronics, electrical engineering, information technology, precision mechanics, physics, and chemistry [56-60]. The application of spectroscopy in biophotonics encompasses fundamental biological research, real-time physiological and biochemical monitoring within organisms, and the development of novel methods for disease diagnosis and treatment control [61].

Compared to SERS-related research projects in Taiwan, this study represents the first published instance of on-site pathogen detection in pigs using SERS, applying SERS to clinical animal disease detection. Furthermore, due to the relatively complex regulatory restrictions in the veterinary system compared to the human medical system, the industry

has shifted its focus towards clinical animal disease detection. Therefore, this study represents an innovative application of SERS from laboratory and human clinical detection to animal disease detection. Ultimately, the findings of this study can be integrated with SERS research results in agriculture, forestry, poultry, aquaculture, and other related fields to establish a Raman spectroscopy database. This database can serve as a platform for rapid detection of diseases in agriculture, forestry, and animal husbandry, facilitating early detection of animal infections and prevailing disease conditions on farms, thereby aiding in disease control and providing necessary certification, quarantine, and epidemic prevention for domestic and international use [62].

Currently, SERS is being increasingly used in the human medical system for the detection of bacterial pathogens and the establishment of bacterial strain Raman spectral analyses [62-66]. These bacterial pathogens include methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* differentiation; *Enterococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*. Additionally, SERS has been applied to detect virus strains (such as different strains of influenza viruses), sample detection of hepatitis C virus, and detection of bacteriophages in the laboratory. In parasite detection, SERS has been applied to the detection of Plasmodium in blood samples [42-47]. Our previous research have been focused on the establishment of bacterial Raman spectra as *Staphylococcus aureus* (ATCC® 6538), *Pseudomonas aeruginosa* (ATCC® 9027), *Salmonella choleraesuis* (ATCC® 12011), *Salmonella typhimurium* (ATCC® 13311), *Actinobacillus pleuropneumoniae* (ATCC® 55454), and *Bordetella bronchiseptica* (ATCC® 19395). However, SERS has not been currently applied to clinical animal disease detection. Therefore, the transition of SERS from laboratory and human clinical detection to animal disease detection represents an innovative application.

In comparing SERS technology with current detection methods (such as PCR and ELISA) in terms of relative advantages and cost-effectiveness, firstly, in terms of sample pretreatment, PCR requires more time (extracting DNA or RNA from samples), while SERS technology and ELISA (blood centrifugation, serum dilution, etc.) should be similar. In terms of detection time, SERS technology takes 30 seconds, PCR depends on the program setting (generally taking 1.5-2.5 hours), and ELISA depends on the test setting (generally taking 2.5-4 hours). In terms of cost-effectiveness, when dealing with a small number of samples, the cost of PCR and SERS technology is similar, but ELISA often incurs additional costs due to the design of the kit, resulting in unused reaction wells being discarded, thus increasing the overall cost. Therefore, considering personnel costs, the total cost is: ELISA > PCR or SERS. Additionally, when dealing with a large number of samples, the cost of ELISA decreases, but the time consumption increases, and personnel costs also increase, resulting in a similar cost to SERS technology when converted. SERS technology, on the other hand, has lower personnel costs due to less time spent on subsequent testing, resulting in a lower total cost when combined with personnel costs: PCR > ELISA or SERS [21].

PCVD is responsible for substantial animal and economic losses to the pig industry. PCV can cause viraemia, pneumonia with abnormal respiratory symptoms, and reduced body weight gain. At present, two main types of PCV, PCV-1 and PCV-2, have been identified. PCV is a highly infectious virus in the pig farms worldwide as pigs are often raised in the areas of high density, the spread of PCVD is difficult to control. Vaccination is a method to prevent PCVD and spread via reducing clinical symptoms, viraemia, and tissue lesions for improving health and performance in pigs [13, 14, 15]. With the intensification and increasing scale of the pig breeding industry, pig disease problems have become increasingly complex, with more cases of mixed infections or secondary infections. Therefore, rapid diagnosis and early prevention of diseases demonstrate their importance. Additionally, the use of antibiotics and vaccines has led to atypical symptoms of bacterial or viral diseases in pigs, often causing significant economic losses for livestock producers. Thus, relying solely on clinical symptoms for rapid diagnosis would be challenging. Strengthening and developing new laboratory diagnostic techniques are currently the primary choice. Therefore, the development of non-invasive real-time spectrum monitoring systems is indeed necessary [54-55].

PCV is constantly evolving to cause new outbreaks. This disease is becoming more difficult prevention with ability to evade vaccine-induced immunity [1, 2, 5]. Therefore, R&D of an effective PCVD vaccine is very important for controlling PCVD outbreaks and preventing economic losses. However, with the intensification and expansion of the domestic livestock industry, harmful substances such as gases, airborne particulate matter, and pathogens in livestock sheds increase with the scale of farming. Therefore, it is necessary to establish new modular pigsty intelligent precision management technology that complies with the breeding environment in our country. Important tasks include as establishing novel modular livestock sheds that comply with the breeding environment in our country and utilize new light-emitting diode (LED) technology for degradation and removal and establishing a harmful substance monitoring system and implementing new visible light LED technology for degradation and removal of harmful substances. The newly developed visible light LED technology has shown promising results in initial tests for degrading harmful substances in pig farms. From the above, it can be seen that this new visible light LED technology has considerable capabilities for removing harmful substances. Additionally, a pathogen diagnostic system using SERS coupled with

Raman spectroscopy has been established for the detection and identification of pathogens. Subsequently, integrating SERS with advanced LED technology and artificial intelligence real-time monitoring systems, a system for purifying and monitoring livestock housing environments will be established in the future goals. This system will be capable of rapidly monitoring harmful substances such as gases, airborne particulate matter, and pathogens in the livestock housing environment. By utilizing the data collected from monitoring, the system will be able to activate new LED technology to degrade harmful substances in the environment in real-time, thereby improving animal health and protecting operational personnel while reducing economic losses in the industry. Taken all our results together, the combination of SERS and Raman spectroscopy has the potential to be applied in livestock and poultry sheds in the future for rapid detection of on-site pathogens, enabling timely initiation of disease control and reducing farmers' economic losses [6, 10, 21-22, 30].

5. Conclusion

The purpose of this study is to utilize SERS in conjunction with surface plasmon resonance effect for application in the field of molecular biology, establishing an animal pathogen detection system. Through this detection system, animal pathogens carried within the body can be quickly and accurately detected, thereby enabling the preliminary control of pathogen transmission and reducing economic losses. In this study, SERS was used to detect the PCV-2 pathogen strain in pig feces and serum. The results showed that both SERS and PCR detection methods were able to detect PCV-2. In the future, this SERS technology can also be applied to the detection of animal infectious diseases, establishing an animal pathogen detection system to assist in the promotion of the development of biotechnology and medical industries.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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