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ORIGINAL ARTICLE

Diagnosis of porcine circovirus type 2 infection with a combination of immunomagnetic beads, single-domain antibody, and fluorescent quantum dot probes

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Abstract The use of a specific antibody conjugated with nanobeads, forming immunomagnetic nanobeads (IMNBs), has been demonstrated to be useful for the capture and detection of viruses. In this study, IMNBs functionalized with a single-domain antibody against the capsid protein (Cap) of porcine circovirus type 2 (PCV2), hereafter denoted as psdAb, were evaluated and used to capture PCV2. Quantum dots (QDs) conjugated with psdAb were used as a fluorescence probe to visualize PCV2 captured by IMNBs. The specificity and sensitivity of this method were further evaluated using common pathogens of pig viral disease and PCV2. To assess its practicality, clinical samples were tested in this study. The results showed that 2.57 ± 0.13 mg Cap or $0.97 \pm 0.064 \times 10^6$ copies of PCV2 particles could be captured by 1 mg of IMNBs in 30 min. This suggests that the IMNBs have the ability to efficiently capture PCV2 with good specificity, as there was no cross-reaction with other pathogens, and with strong sensitivity, with a detection limit as low as 10^3 copies/ml of PCV2 particles. Moreover, PCV2 in inguinal lymph node, lung, spleen, serum, and fecal samples was successfully detected by IMNBs. The results demonstrate that this method is promising for the rapid and effective detection of PCV2 in complex clinical samples.

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Introduction

Porcine circovirus type 2 (PCV2) is the major infectious pathogen of post-weaning multisystem wasting syndrome (PMWS) [2, 3]. The unique features of PMWS are wasting and lymphoid depletion, which indicate deep involvement of the pig's immune system in the pathogenesis of PCV2 infection. In addition, PCV2 has also been found to be the primary causative pathogen of several syndromes collectively known as porcine circovirus-associated disease (PCVAD) [17, 18, 35]. Recent studies have suggested that there are several pivotal factors in the development of PCVAD [39]. PCV2 infection can result in a subclinical infection or PCVAD, depending on a combination of these factors. With the commercialization of successful vaccines [4], the incidence of PMWS and PCVAD has decreased. However, PCV2 can still be found in pigs worldwide. Given the nature of its pathogenesis, the existence of PCV2 in pigs is still a potential threat to the pig industry.

PCV2, a member of the genus *Circovirus*, family *Circoviridae*, is a non-enveloped, single-stranded, circular DNA virus with a genome of 1767–1768 nucleotides [1]. The PCV2 genome contains two major open reading frames (ORFs), namely, ORF1 and ORF2. ORF1 is essential for viral DNA replication, whereas ORF2 encodes a capsid protein (Cap) that is involved in the host immune response [29]. Based on sequence analysis, PCV2 isolates can be further divided into four main genotypes (PCV2a-d). A shift in genotype prevalence from PCV2b toward PCV2d has been observed, but the PCV2b genotype currently still predominates worldwide [45]. So far, the most

widely used diagnostic test is the polymerase chain reaction (PCR) [21]. However, PCR has not been shown to be specific and/or sensitive enough for the detection of low virus loads in complex biological samples [16, 20]. The reliability of PCR methods is also compromised by with nonspecific reactions and nucleases that can degrade samples. PCR methods require multiple operating steps and are time-consuming. Rapid and accurate diagnostic methods are essential for detection of virus in clinical samples, and the early and accurate detection of PCV2 is particularly important for the control and elimination of PCVAD.

As a nanomaterial, superparamagnetic beads composed of assemblies of iron oxide nanobeads have unique properties in that they are easily conjugated with recognition units, easily bound to analytes, conveniently manipulated, suitable for a range of chemical separation, stable in the long-term, and available through a number of synthetic strategies and commercial sources [38, 51]. In view of these unique properties, superparamagnetic beads have been widely used in biomedical studies and detection of diseases associated with bacteria and viruses [27, 28, 30, 37]. Those applications have shown that the used of superparamagnetic beads has the advantages that it allows multiplexing, provides high sensitivity, reduces the time required for analysis, and permits control of selectivity. When superparamagnetic beads are used for the detection or separation of bacteria or viruses, recognition units are normally required. Monoclonal antibodies (mAbs) [8, 26, 51] and peptides [34, 37] that can specifically bind to the target virus have been conjugated to the surface of superparamagnetic beads as recognition units for the detection or separation of targets. However, the stability of mAbs may be reduced when mAb-conjugated superparamagnetic beads are applied under different extreme conditions.

Single-domain antibodies (sdAbs) are derived from the variable region of heavy-chain antibodies found in camelids and sharks [22, 23]. The unique properties of sdAbs include low molecular weight (15 kDa), easy expression in bacteria, and a degree of thermostability that is not observed with conventional antibodies. Remarkably, sdAbs are often able to regain functional activity by refolding to their native structure after denaturation under different extreme conditions [14, 15]. Thus, sdAbs may be more suitable for use as recognition binders for detection of pathogenic microorganism or separation of complex clinical samples.

Semiconductor nanocrystal quantum dots (QDs) have been widely used in biomedicine as fluorescent probes due to their unique optical properties, such as a wide excitation spectrum, size-tunable fluorescence emission, high quantum yields, and reduced photobleaching. Normally, QD-based fluorescent probes are produced by conjugating QDs with recognition molecules. Recent research has shown that QD-based probes can be successfully functionalized with recognition molecules such as peptides [25], antibodies [19, 40], or nucleic acids [6, 41]. QD-based fluorescent probes have been used in a variety of laboratory applications, such as the detection of cancer cells, bacteria, and viruses [11, 13, 33].

In earlier work, three clones of a single-domain antibody specific for PCV2 (psdAb) that displayed excellent binding affinity and high specificity for the PCV2 Cap protein, were selected from a VHH library derived from a Bactrian camel [46]. In this study, two of the clones, namely, psdAb-c1 and psdAb-c4, were used to produce immunomagnetic nanobeads (IMNBs) and a QD-psdAb probe, respectively. A robust and sensitive method for detection of PCV2 was developed by using IMNBs for the capture of PCV2 particles and a QD-psdAb probe to produce a visual signal. The robustness of this method is demonstrated by its ease of operation, good specificity, and sensitivity in detecting PCV2 in complex clinical samples.

Materials and methods

Ethics statement

Lungs, spleens, inguinal lymph nodes, sera, and fecal samples were collected from pigs at a local pig farm and a slaughterhouse in China. The procedures for sampling were performed according to the guidelines of the Chinese Academy of Agricultural Sciences, Lanzhou Veterinary Research Institute. This study did not raise any ethical issues.

Preparation of PCV2 Cap protein and PCV2 virus

Recombinant Cap of PCV2 was expressed in *Escherichia coli* (*E. coli*) and purified using Ni^{2+} affinity resins. The concentration was determined by spectrophotometry at 280 nm using a NanoDrop2000 (Thermo-Fisher, Wilmington, DE, USA) as described previously [48]. PCV2 virus (GenBank accession number FJ948167) was propagated in the porcine kidney 15 cell line (PK-15), which contained 1×10^6 PCV2 genomic copies/ml as determined by real-time quantitative PCR (qPCR) as described previously [32]. The virus was aliquoted at 5 ml/tube and stored at −70 °C to be used as a positive control.

Production of IMNBs

IMNBs were obtained by incubating EDC (1-ethyl-3-[3-dimethyl aminopropyl] carbodiimide hydrochloride)- and NHS (*N*-hydroxy succinimide)-activated superparamagnetic nanobeads (200 nm, 3 mg/ml, purchased from

Ademtech SA, Pessac, France) with 200 μg of psdAb-c1 (1 mg/ml) in 1 ml of 0.01 M phosphate-buffered saline (PBS, pH 7.4) for 2 h at 37 °C with a gentle shaking at 120 rpm. They were then blocked with 0.1 % (m/v) bovine serum albumin (BSA) for 30 min at 37 °C. The IMNBs were separated with a magnetic scaffold and resuspended in 1 ml of PBS and stored at 4 °C for future use.

psdAb conjugation of QDs

Thioglycolic-acid-modified CdSe/ZnSe QDs (605 nm), synthesized by Wuhan Jiayuan Quantum Dots Co. Ltd. (Wuhan, China), were conjugated with psdAb-c4 as described previously [49]. The water-soluble QDs were dissolved in 50 mM borate (pH 8.4) containing 50 ml of EDC and covalently coupled with psdAb-c4 (dissolved in 50 mM borate, pH 8.4) at room temperature for 4 h with gentle shaking at 120 rpm. Conjugates were purified by centrifugation at 6000 *g* for 10 min, and the supernatant was dialyzed in a dialysis tube (MW, 50 kDa) in borate buffer (0.5 M $Na_2B_4O_7$, 0.2 M H_3BO_3, pH 8.4) at 4 °C to retain molecules with a molecular weight of 50 kDa and to remove unreacted psdAb and impurities. The conjugates were then concentrated by ultrafiltration until the appropriate concentration was reached. The fluorescence spectra and UV-visible absorption spectrum of the QD-psdAb were produced using a spectrophotofluorometer (Perkin Elmer, America) and an ultraviolet-visible spectrophotometer (SHIMADZU, Japan). The QD-psdAb probes were stored at 4 °C for future use.

Binding of IMNBs to PCV2 Cap protein

Binding of IMNBs to Cap was detected using an enzyme-linked immunosorbent assay (ELISA) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). IMNBs (10 μl, 3 mg/ml) were incubated with recombinant Cap (50 μl, 1.35 mg/ml) at 37 °C for 1 h in a tube. Then, IMNB-Cap composites were separated with a magnetic scaffold to remove the suspension and washed four times with PBST (0.1 % Tween-20 in PBS). A total of 100 μl of PCV2-positive porcine serum diluted at 1:50 (VMRD, Washington, USA) was added and incubated for 1 h at 37 °C. After washing four times, the bound antibody was detected by adding 100 μl of horseradish peroxidase (HRP)-conjugated anti-pig IgG diluted at 1:40 000 (Sigma-Aldrich, NY, USA). The color reaction was developed by adding 100 μl of tetramethyl benzidine (TMB) substrate and stopped by adding 100 μl of 2 M of H_2SO_4. The OD_{450} value was measured using a microplate reader (Bio-Rad, CA, USA). A tube without Cap was used as a negative control. The separated IMNB-Cap composites were detected directly by SDS-PAGE.

Binding of IMNBs to PCV2 particles

The binding of IMNBs to PCV2 was detected directly using a transmission electron microscope (TEM) and a Zetasizer Nano ZS series system (ZEN 3600; Malvern Instruments Ltd., Worcestershire, UK). Approximately 10^6 PCV2 particles were mixed with 3 mg of IMNBs per ml in 200 μl of PBS (PH 7.2). After 1 h of incubation at 37 °C, the IMNB-virus complexes were separated and washed four times with PBST. Then, one part of the IMNB-virus complexes was adsorbed onto a copper grid (300 mesh, Pelco, CA, USA) at room temperature. After staining with 3 % phosphotungstic acid, images were recorded on a JEOL 2010 TEM operated at an acceleration voltage of 100 kV. The other part of the IMNB-virus complexes and IMNBs was analyzed by measuring dynamic light scattering. The diameters of the IMNBs-virus complexes and IMNBs were calculated using Dispersion Technology Software version 4.20.

Capture efficiency of IMNBs

The optimal capture time was determined by incubating the IMNBs with Cap. IMNBs (0.05 mg) were incubated with Cap (0.2 mg) for 5, 10, 15, 20, 25, 30, 40, and 60 min. Then, the IMNB-Cap complexes were separated using a magnetic scaffold. The concentration of Cap in the supernatants at different reaction times was recorded, and the capture rates were calculated by measuring the decrease in the concentration of Cap in the supernatants.

The maximal capture dose of Cap was determined by incubating 0.1 mg of IMNBs with twofold dilutions containing from 3.2 mg to 0.05 of mg Cap at 37 °C for 30 min. Then, the IMNB-Cap complexes were separated using a magnetic scaffold, and the concentration of Cap in the supernatant was determined using a NanoDrop2000. The capture rates were calculated by determining the decrease in the concentration of Cap.

For the detection of the maximal capture dose of PCV2 particles, 10^6 PCV2 particles were incubated with IMNBs that were serially diluted twofold from 1.6 mg to 0.05 mg at 37 °C. After incubation for 30 min, the IMNB-virus complexes were separated using a magnetic scaffold and washed four times with PBST. Then, the IMNBs-virus complexes were used for the extraction of genomic DNA of PCV2 using a DNA Extraction Kit (QIAGEN, Hilden, Germany). The number of copies of PCV2 DNA captured by IMNBs was determined by qPCR using a PCV2-specific primer and a plasmid standard.

PCV2 detection protocol based on IMNBs and QD-labeled Nanobody

IMNBs (0.1 mg) were mixed with lysates from PCV2-infected PK-15 cells (0.5 ml) and incubated at 37 °C for

30 min. After separation and washing three times with PBST, the IMNB-virus complexes were resuspended in 200 µl of PBS and incubated with 100 µl of QD-psdAb probe (diluted 1:200 in PBS) at 37 °C for 30 min. After another separation and washing four times, the IMNB-virus-QD complexes were resuspended in 100 µl of PBS, and a fluorescence image was obtained by confocal laser scanning microscopy (Leica, Germany) at 605 nm wavelength.

As a parallel control, the IMNBs-virus complexes stained with the QD-psdAb probe were detected using an indirect immunofluorescent assay (IFA) with a positive porcine antiserum against PCV2 and fluorescein isothiocyanate (FITC)-conjugated goat anti-pig IgG (BSZH, Beijing, China). The fluorescence image was obtained by confocal laser scanning microscopy at the wavelengths of 605 nm and 488 nm. IMNBs were also incubated with PBS, which was used as a negative control.

Specificity and detection limit

Recombinant structural proteins of several common viral pathogens of pigs, including classical swine fever virus (CSFV) E2 protein [47], PCV1 Cap, and porcine reproductive and respiratory syndrome virus (PRRSV) GP5 protein [12], were expressed in *E. coli* in our laboratory and used to evaluate the specificity of the method using immunomagnetic beads and QD-labeled Nanobodies. Those recombinant proteins were incubated with IMNBs and visualized by QD-psdAb as described above.

PCV2 propagated in PK-15 cells was used to determine the detection limit. The IMNBs were incubated with a tenfold serial dilution of PCV2 in the concentration range of 2.7×10^6 copies/ml to 2.7×10^1 copies/ml. Bound virus was visualized using a QD-psdAb probe.

Detection of PCV2 in clinical samples

Lungs, spleens, inguinal lymph nodes, sera, and fecal samples were collected from fifteen pigs. Five of the pigs showed symptoms of respiratory distress, inappetence, diarrhea, and finally, death. Ten of the pigs, which had not shown any clinical symptoms, were from a slaughterhouse. Small blocks of organ and tissue were ground in sterile PBS (pH 7.4) to produce a 10 % (W/V) suspension. The tissue suspension was clarified by centrifugation at 2000 *g* for 15 min. A small quantity of fecal material collected from each pig was mixed with 1 ml of sterile PBS. The serum was separated from blood by centrifugation at 2000 *g* for 10 min. All samples were stored at −80 °C until testing.

For virus detection, IMNBs (100 µl, 3 mg/ml) were incubated with 1 ml of tissue, serum, or fecal suspension at 37 °C for 30 min. Captured viruses were labeled with the QD-psdAb probe. In order to further confirm the validity of the result, total DNA was extracted from the IMNBs complexes using a DNA Extraction Kit (QIAGEN, Hilden, Germany). The Cap gene of PCV2 was detected by PCR using a specific primer [50].

Results

Binding of IMNBs to PCV2

The binding of IMNBs to the Cap protein was analyzed by SDS-PAGE and ELISA. A protein of 45 kDa was observed in the IMNBs, corresponding in size to the Cap protein (Fig. 1A). In addition, in the ELISA, the IMNBs and Cap samples gave higher OD_{450} values than the control (Fig. 1B). These results demonstrate that the IMNBs can directly bind with Cap.

TEM was then used to examine the binding of IMNBs to PCV2 particles. As shown in Fig. 1C, particles with diameters of approximately 20 nm, similar to the average diameter of PCV2 particles, were found around the IMNBs. In addition, the sizes of the nanobeads (198 ± 3.2) and IMNBs-PCV2 complexes (255.6 ± 2.1) were consistent with the predicted size distribution (Fig. 1D).

Capture efficiency

The time required for IMNBs to bind to the targets was determined by comparing the capture rates. As shown in Fig. 2A, when Cap was in excess, the capture rate did not increase when the incubation times were longer than 30 min. We therefore used 30 min as the incubation time in the subsequent assay.

Different concentrations of Cap and approximately 10^6 PCV2 particles/mL were used for the analysis of the maximal capture dose of IMNBs. When the IMNBs were saturated, the bound Cap and PCV2 particle doses were calculated. Approximately 2.57 ± 0.13 mg of Cap (Fig. 2B) or $0.97 \pm 0.064 \times 10^6$ copies of PCV2 particles (Fig. 3) were bound per mg of IMNBs.

Rapid detection of PCV2 based on IMNBs and a QD-psdAb probe

The principle of PCV2 detection based on IMNB separation and a QD-psdAb probe is shown in Fig. 4A. Absorption spectra of QD-psdAb probe were acquired on a Perkin Elmer LS-55 UV/vis spectrometer, and the particles demonstrated the expected optical properties, including absorption and emission of the QD-psdAb probe (Fig. 4B).

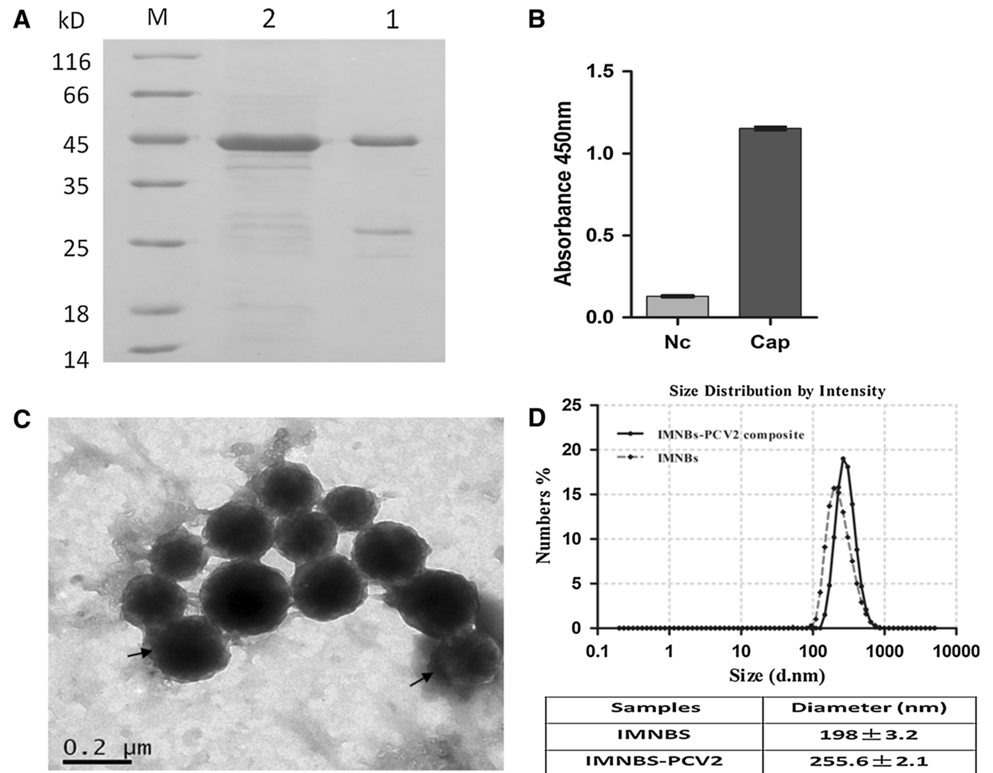

Fig. 1 Binding of IMNBs to Cap and PCV2 particles. Cap protein and lysates of PCV2-infected PK-15 cells was incubated with IMNBs at 37 °C for 1 h. **A.** Visualization of the bound Cap protein by SDS-PAGE. **B.** Detection of the bound Cap protein by ELISA. **C.** Detection of the bound PCV2 particles by TEM. The dark spots are IMNBs, and the small spots (indicated by arrows) around the IMNBs are viruses. **D.** Characterization of IMNBs by dynamic light scatting. The hydrated particle sizes of IMNBs and IMNBs-virus complexes were determined by dynamic light scattering

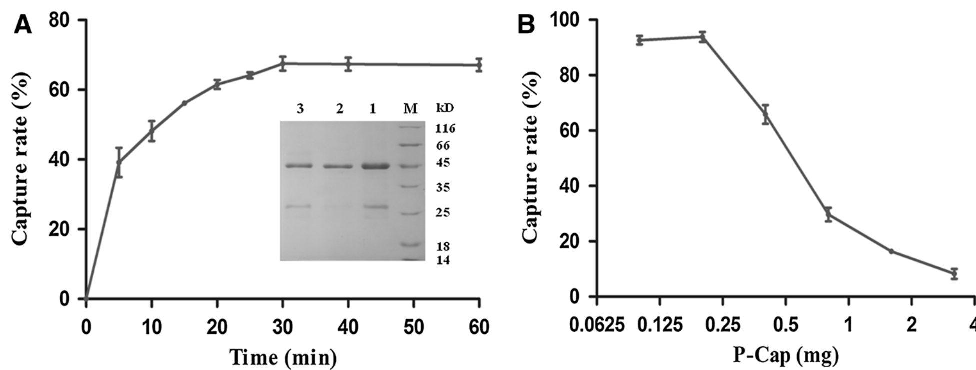

Fig. 2 Capture efficiency of IMNB binding to Cap. The Cap protein was incubated with IMNBs for different lengths of time. The capture rates were determined by measuring the decrease of Cap in the supernatant. **A.** Plot of the time-dependent change in the capture rate at an IMNB dosage of 0.5 mg. **Inset:** SDS-PAGE showing the total amount of Cap used (lane 1), Cap captured by IMNBs (lane 2), and Cap in the supernatant (lane 3) after 30 min of incubation. **B.** Plot of capture rate versus different doses of Cap with the same amount of IMNBs (0.1 mg). The data are expressed as the mean ± SD of three repeats

PCV2 particles were separated using IMNBs, identified using a QD-psdAb probe, and captured as an image by IFA using a confocal laser scanning microscope at wavelengths of 605 nm and 488 nm (Fig. 4C). The QD-psdAb probe allowed the same PCV2 particles to be visualized by IFA. This result demonstrates that the QD-psdAb probe bound specifically to the PCV2 particles. In addition, the negative control also demonstrated that the QD-psdAb probe could not bind directly to IMNBs (Fig. 4D).

Specificity and detection limit

For analysis of the specificity of the fluorescence approach, several common porcine virus antigens, including CSFV E2, PCV1 CAP, and PRRSV GP5, were used as controls. Typical fluorescence visualized by the QD probe was easily detected in PCV2-positive samples, whereas no obvious QD fluorescence was found in the PCV2-negative samples of CSFV, PCV1, and PRRSV

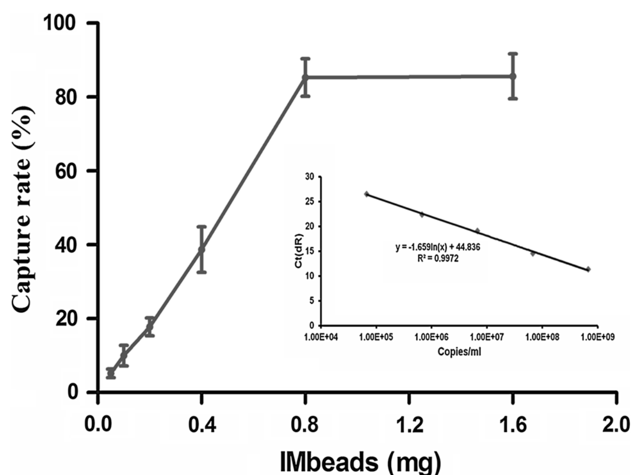

Fig. 3 Efficiency of capture of PCV2 particles by IMNBs. Approximately 10^6 particles of PCV2 were incubated with different doses of IMNBs, the viral genomic DNA was extracted from the detached IMNB-virus composites, and the number of genomic copies of PCV2 was determined by qPCR. **Inset:** Calibration curve used for PCV2 copies calculated by qPCR. The data are expressed as the mean ± SD of three repeats

(Fig. 5). These results demonstrate the specificity of this assay for PCV2.

For determination of the detection limit, serially diluted PCV2 virus was detected by this method. Typical QD fluorescence was not found at concentrations below 2.7×10^3 particles/mL. This detection limit is similar to that of the previously reported real-time PCR method [32].

Detection of virus in clinical samples

Lungs, spleen, inguinal lymph nodes, sera, and fecal samples from fifteen pigs, including healthy pigs and those with suspected PMWS, were evaluated using this method. The results showed that samples of eight spleens and inguinal lymph nodes, six lungs, two sera, and three fecal samples were PCV2 positive (Table 1), which was confirmed by PCR. The method based on IMNBs and a QD-probe is therefore suitable for rapid detection of PCV2 in complex clinical samples.

Discussion

PCV2 continues to be of economic importance in the global swine industry, even though the recent availability of vaccines has decreased the economic impact of PCV2 in swine herds. Current studies have demonstrated that PCV2 can be transmitted and persistent infection can be found in multiple tissues, sera, and secretions of infected pigs [7, 10, 36]. The main objective of this study was to develop a detection method based on IMNBs and QDs for the rapid detection of PCV2 in complex clinical samples.

Four genotypes of PCV2 (PCV2a-d) have been documented worldwide, and PCV2b is currently the predominant genotype [45]. For the detection of pandemic PCV2 strains, psdAbs capable of binding to the Cap protein of PCV2b [46] were used to produce IMNBs and a QD-psdAb probe.

Antibody-conjugated nanobeads have been used for the capture and separation of pathogens and cells [28, 44]. Superparamagnetic nanobeads were used in this study to capture PCV2 because of their effectiveness and ease of use. The first step of conventional detection methods for clinical samples is usually the extraction of DNA or RNA for PCR or histotomy for immunohistochemistry. Compared with these other methods, the use of nanobeads reduces the number of operating steps and instruments as well as the amount of time required, since only a magnetic scaffold is needed, and the test can be completed in several minutes.

To achieve target-specific detection of PCV2, the selected antibody must exhibit both binding affinity and specificity for the desired antigen. The Cap protein encoded by ORF2 is a major structural protein of PCV2 that is the principal carrier of type-specific epitopes involved in host immune responses [31]. Thus, the Cap protein was used as a key marker for PCV2 detection in this study. Conventional antibodies, such as monoclonal antibodies (mAbs) can be used for the detection and separation of viruses [51]. However, taking into consideration the stability of complex clinical samples in varying extreme conditions, mAbs may not be the best choice. sdAbs have the important properties of withstanding harsh physiochemical treatments, maintaining their solubility under certain nonphysiological conditions, and regaining their activity following exposure to chemical denaturants [15, 42]. Thus, we used a PCV2-specific sdAb as a binder, conjugated with superparamagnetic nanobeads.

qPCR is widely used for PCV2 detection, but marked variation in qPCR detection limits has been reported [9, 20, 24]. The potential diagnostic PCV2 load threshold is strongly dependent on the laboratory and particular technique used. Thus, the enrichment of PCV2 in samples with a low virus load is important for the accurate detection of PCV2. In this study, IMNBs and a magnetic scaffold were used for the capture and enrichment of PCV2 in clinical samples. To determine the capture efficiency and maximal possible captured dose of IMNBs, incubation experiments were performed using PCV2 particles. Approximate $0.97 \pm 0.064 \times 10^6$ copies of PCV2 particles were captured by 1 mg of IMNBs, indicating that IMNBs are suitable for the capture and enrichment of PCV2.

QDs are ideal fluorophores and have been used for numerous biological and biomedical imaging applications [5, 43]. They can be conjugated with different biological probes, particularly antibodies. In order to simplify the

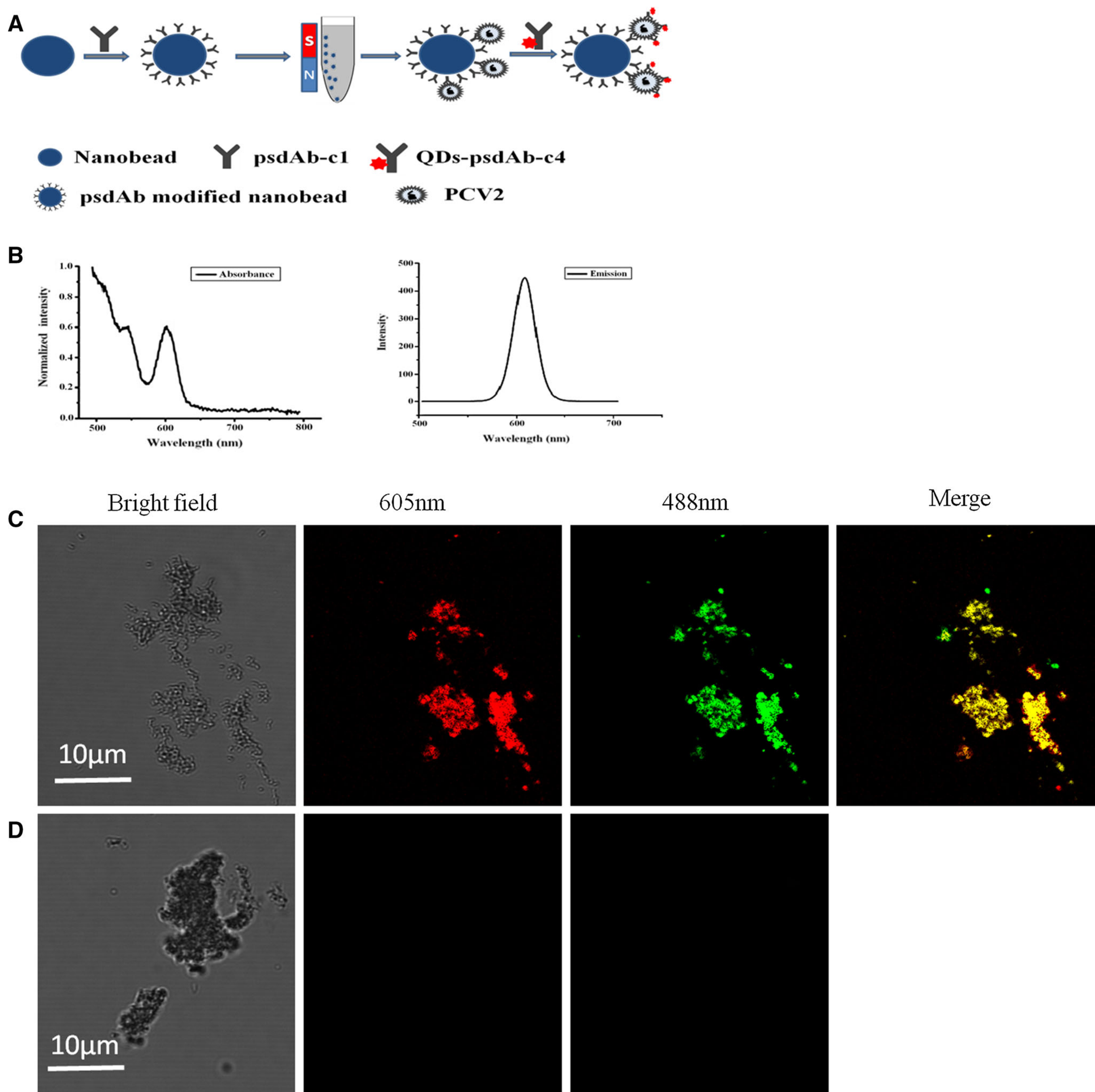

Fig. 4 Fluorescence approach for PCV2 detection based on IMNB separation. The captured virus was visualized using a QD-conjugated psdAb-c4 probe. **A.** Principle of the fluorescence approach. **B.** Emission spectra of QD-labeled psdAb-c4. **C.** Captured PCV2 detected using the QD-conjugated psdAb-c4 probe and indirect immunofluorescence based on anti-PCV2 pig antibodies and FITC-conjugated anti-pig antibody. The images were viewed in a bright field at 488 nm, and 605 nm wavelength by confocal microscopy. **D.** IMNBs that were not incubated with PCV2, used as a negative control

detection process, another clone of psdAb selected previously [46] was conjugated with QDs and used as a fluorescence probe for visualization. Thus, PCV2 captured by IMNBs can be visualized directly using a psdAb probe. To determine the specificity and sensitivity of this method, several antigens of common pig pathogens and serially diluted PCV2 particles were tested. The method showed good specificity and high sensitivity (10^3 copies/ml), which was similar to that of qPCR [32]. Furthermore, to evaluate the applicability of the fluorescence method for detection of virus in clinical samples, 75 samples, including serum, tissues, and feces, were tested using our method. PCV2 captured by IMNBs was also detected by PCR. The results showed that 27 samples were PCV2 positive, which was in

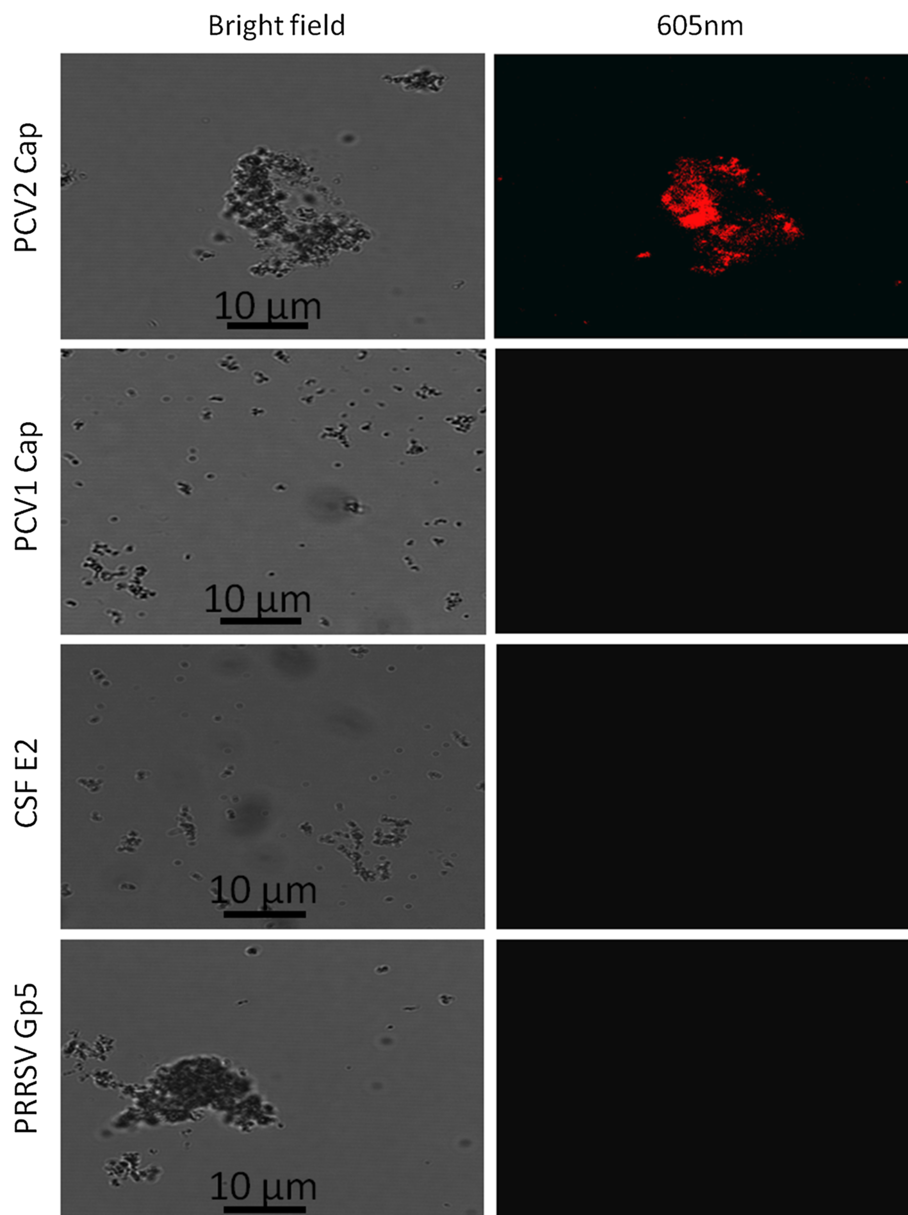

Fig. 5 Assay specificity. Recombinant E2 protein of classical swine fever virus (CSF E2), Cap protein of the porcine circovirus type 1 (PCV1 Cap), GP5 protein of porcine reproductive and respiratory syndrome virus (PRRSV GP5), and PCV2 Cap protein were used to assess the specificity of the fluorescence approach

Table 1 Detection of virus in clinical samples

Sample source	Number positive*	Number negative*
Lung	6	9
Spleen	8	7
Inguinal lymph node	8	7
Serum	2	13
Feces	3	12
Total	27	48

Clinical samples collected from a pig farm and a slaughterhouse were detected using this method, and the results were confirmed by PCR

* The number of positive and negative samples detected using IMNBs and a QD-psdAb probe was consistent with the result obtained using PCR

accordance with PCR detection. The results demonstrated that the fluorescence method is suitable for use in the rapid detection of PCV2 in complex clinical samples.

Conclusion

In this study, a rapid and sensitive method for PCV2 detection was developed by using IMNBs for the capture of PCV2 and fluorescent QDs-psdAb probes for detection. This method is specific and sensitive for PCV2 detection. Furthermore, the use of IMNBs and QDs-psdAb probes greatly simplified PCV2 capture and detection. Validation of the assay using clinical samples demonstrated the

potential of this method for rapid and effective detection of PCV2 in complex clinical samples.

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Conflict of interest None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence the content of this paper.

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