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Letter

A ferritin nanoparticle-displayed spike protein-based ELISA for serological surveillance and immune evaluation of porcine deltacoronavirus

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Dear Editor,

Porcine deltacoronavirus (PDCoV), a member of the genus *Deltacoronavirus*, is a significant enteric pathogen that induces acute watery diarrhea, vomiting, and high mortality in neonatal piglets (Jung et al., 2016; Yao et al., 2025). After porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV), porcine deltacoronavirus (PDCoV) has emerged as a major threat to the global swine industry. Recent molecular surveillance indicates a nucleic acid positivity rate of approximately 6%–10% in clinical samples (Wu et al., 2026; Yao et al., 2025). Notably, its broad tissue tropism and potential for cross-species transmission presents a substantial risk to both veterinary and public health (Hu et al., 2024). While PCR-based assays are standard for acute diagnosis, serological testing remains indispensable for assessing infection dynamics and vaccine-induced immunity. However, current reference methods, such as the immunofluorescence assay (IFA) and virus neutralization test (VNT), are labor-intensive and unsuitable for high-throughput screening (Paudel et al., 2014). Given the absence of standardized commercial ELISA kits in China, developing a rapid, sensitive, and cost-effective serological tool is essential for large-scale epidemiological investigations and vaccine evaluation. Among the structural proteins of PDCoV, the spike (S) glycoprotein is the primary determinant of host cell entry and the dominant target for neutralizing antibodies, making it the most promising antigen for serological diagnostics and vaccine development (Chen et al., 2020; Li et al., 2023). While S protein-based iELISA has been successfully established for other porcine coronaviruses like PEDV, their systematic application for PDCoV remains underdeveloped (Ma et al., 2025). Notably, maintaining the native trimeric conformation of the S protein is crucial for maximizing diagnostic sensitivity, as it preserves key conformational epitopes.

Despite the increasing prevalence of PDCoV and its substantial impact on the swine industry, sensitive and reliable serological assays for large-scale surveillance and vaccine evaluation remain limited. In particular, conventional iELISA antigens produced in prokaryotic expression systems often lack authentic post-translational modifications and native conformational epitopes, which may compromise diagnostic performance. To address these limitations, this study employed a mammalian expression system to produce the PDCoV spike ectodomain (S-ECD) protein and incorporated a ferritin-based self-assembling nanoparticle platform to enhance antigen presentation and preserve conformational epitopes (Ding et al., 2026). Based on this strategy, an iELISA was developed and systematically evaluated for its potential application in PDCoV serological detection, epidemiological surveillance, and vaccine immunogenicity assessment.

To obtain high-quality soluble recombinant spike (S) protein, the S-ECD was expressed using a mammalian expression system. As shown in the schematic workflow (Fig. 1A), the expression vector pMGT was constructed by fusing the S-ECD sequence with a ferritin tag to facilitate self-assembly into nanoparticles. A Glycine-Serine (GS) linker was incorporated to provide structural flexibility between the viral antigen and the ferritin scaffold.

The recombinant S protein was purified via a Superose 6 Increase 10/300 GL SEC column (Supplementary Fig. S1). As shown in Fig. 1B, the recombinant S protein appears at approximately 200 kDa on the SDS-PAGE, which is larger than its predicted molecular weight attributable

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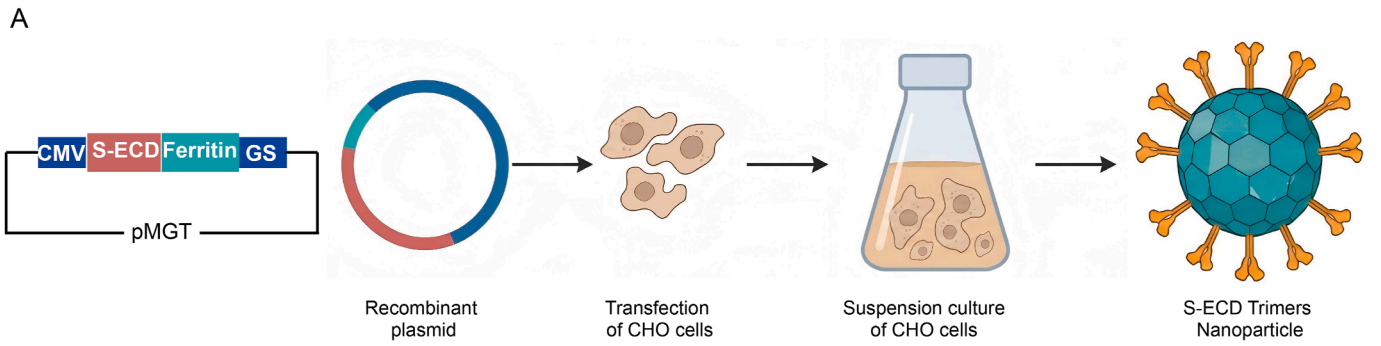

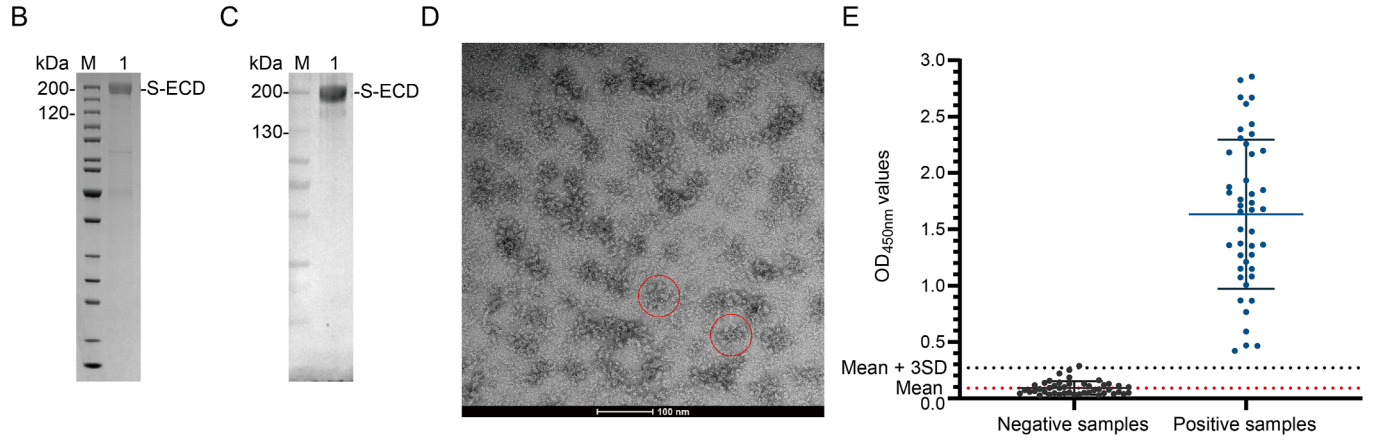

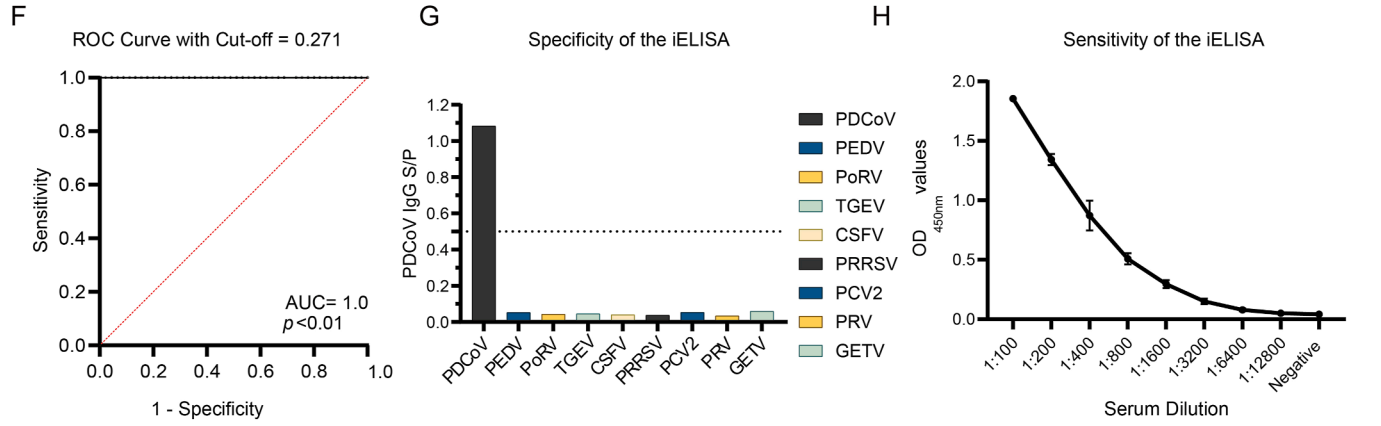

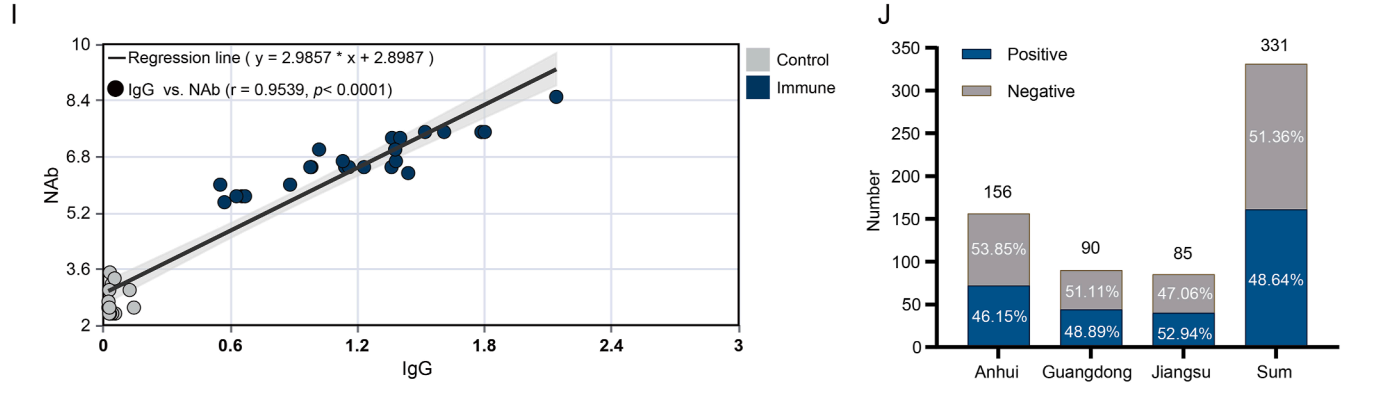

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to extensive N-glycosylation of the S-ECD, and the absence of additional bands confirmed the high purity of the recombinant protein. Subsequent Western blot (WB) analysis using PDCoV-positive porcine serum revealed a specific band at 200 kDa, further confirming the identity and the potent antigenicity of the expressed S protein (Fig. 1C). Finally, Transmission electron microscope (TEM) examination of the purified S-ECD-ferritin fusion protein demonstrated the formation of hollow, cage-like nanoparticles decorated with distinct trimeric spikes on the surface, validating ferritin-mediated self-assembly (Fig. 1D).

The optimal working concentrations of antigen and serum were determined by checkerboard titration based on the highest P/N ratio (Supplementary Table S1). The results indicated that the S-based iELISA performed best with a coating antigen concentration of 2.0 μg/mL and a serum dilution of 1:100, yielding a peak P/N ratio of 23.5 (mean OD_{450nm} of 1.081 for positive sera and 0.046 for negative sera). Optimization of the assay conditions identified 10% CN as the most effective blocking buffer (Supplementary Fig. S2A), and the optimal dilution of the HRP-conjugated secondary antibody was 1:20,000 (Supplementary Fig. S2B). The optimal coating time is 15 h at 4 °C (Supplementary Fig. S2C), with an optimal blocking duration of 4 h at 37 °C (Supplementary Fig. S2D). The optimal incubation time for both serum samples and the secondary antibody was determined to be 30 min at 37 °C (Supplementary Fig. S2E and 2F). Finally, the optimal substrate reaction time with horseradish peroxidase (HRP) and tetramethylbenzidine (TMB) was determined as 15 min at 37 °C (Supplementary Fig. S2G).

To determine the positive cut-off value for the iELISA, a total of 90 well-characterized serum samples were analyzed, comprising 45 seronegative samples (collected from newborn, colostrum-deprived piglets, ensuring the total absence of PDCoV antibodies) and 45 seropositive samples (collected post-immunization) (Fig. 1E). The mean OD_{450nm} value of the PDCoV-negative control sera was 0.092, with a SD of 0.0596. Therefore, the cut-off value was calculated as the mean OD value of the negative control sera plus three times the SD (Cut-off = $\bar{x}$ neg + 3 × SD), resulting in a threshold OD_{450nm} value of 0.271, above which samples were considered PDCoV-seropositive. To further validate the diagnostic performance of the assay, a receiver operating characteristic (ROC) curve analysis was performed (Fig. 1F). The result demonstrated an area under the curve (AUC) of 1.00, indicating excellent diagnostic accuracy. This idealized performance is attributed to the distinct biological backgrounds of the reference cohorts: the negative group consisted of newborn, colostrum-deprived piglets (ensuring a zero-antibody baseline), while the positive group comprised high-titer immune sera. At the fixed cut-off value of 0.271, the iELISA achieved a sensitivity of 100% and a specificity of 97.78%, confirming the reliability of this threshold for distinguishing PDCoV-seropositive from seronegative samples.

To evaluate the specificity of the iELISA, ELISA-confirmed positive antisera against other common swine viruses were evaluated. The OD_{450nm} values of these sera for PEDV, porcine rotavirus (PoRV), TGEV, classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), pseudorabies virus (PRV), and Getah virus (GETV) were all below the established cut-off value (Fig. 1G), demonstrating that the S-based iELISA exhibited no cross-reactivity with these samples. Analytical sensitivity was evaluated by testing twofold serial dilutions of PDCoV-positive serum samples, starting from an initial dilution of 1:100 in PBS. As shown in Fig. 1H, the highest dilution yielding a positive signal was 1:1600 (OD_{450nm} = 0.357 ± 0.017), indicating high analytical sensitivity for PDCoV antibody detection. Furthermore, the reproducibility of the iELISA was evaluated using PDCoV-positive sera with varying antibody titers and a PDCoV-negative serum sample, with each tested in triplicate across three independent assays. The intra-assay and inter-assay coefficients of variation (CV) ranged from 1.93% to 3.47% and 2.75%–6.01%, respectively (Supplementary Table S2). All CV values were well below the 10% threshold, confirming the excellent precision and stability of the established iELISA.

To evaluate the diagnostic performance of the iELISA, a comparative study was performed. A total of 78 serum samples collected two weeks after the second immunization with the inactivated PDCoV vaccine and 18 PDCoV-negative serum samples were tested using both the iELISA and IFA. As shown in Supplementary Fig. S3, an anti-N monoclonal antibody confirmed successful viral infection in cells, with no non-specific reactions observed in negative porcine sera. The iELISA and IFA identified 100% (78/78) and 97.44% (76/78) of the positive samples, respectively. The overall agreement between the two methods was 93.75%, with a positive coincidence rate of 94.87% and a negative coincidence rate of 88.89% (Supplementary Table S3), indicating an excellent level of agreement and demonstrating the strong potential of the iELISA for clinical diagnostics.

As shown in Fig. 1I, a significant correlation was observed between PDCoV-specific IgG levels measured by iELISA and neutralizing antibody (NAb) titers ($r = 0.95$, $P < 0.001$). This strong correlation indicates that the assay reliably reflects neutralizing activity, making it a valuable tool for evaluating vaccine-induced immunity.

Finally, the iELISA was applied to 331 field serum samples collected from diarrheic pigs across various regions of China (Jiangsu, Guangdong, and Anhui provinces). The results revealed an overall seropositivity rate of 48.64% (161/331). Regional analysis showed widespread circulation of PDCoV in east and south China, with high seroprevalence in Jiangsu (52.94%), Guangdong (48.89%), and Anhui (46.15%) (Fig. 1J). Considering that none of the sampled pigs had been vaccinated against PDCoV and that PDCoV RNA was also detected in herds showing clinical symptoms, these findings strongly suggest that PDCoV is prevalent and widely circulating among swine populations in these regions.

While vaccination is a premier strategy for controlling swine enteric coronaviruses, the current lack of commercial options renders serological surveillance indispensable for monitoring viral dynamics and evaluating S-protein-targeted vaccine candidates. (Gruell et al., 2022; Tang et al., 2021). Moreover, most subunit vaccines, mRNA vaccines, and other vaccine strategies targeting PDCoV have been designed based on the S protein (Tang et al., 2021). In this study, we developed an S-protein-based iELISA utilizing a ferritin-nanoparticle platform expressed in a stable CHO cell line. Compared to transient expression or prokaryotic systems, our CHO-based platform ensures authentic post-translational

Fig. 1. Establishment, evaluation, and application of an S-protein-based indirect ELISA for detecting PDCoV antibodies. **A** Schematic diagram of the recombinant porcine deltacoronavirus (PDCoV) spike (S) protein used as the coating antigen for the indirect ELISA (iELISA). **B, C** SDS-PAGE (**B**) and Western blot (**C**) analysis confirming the purity and immunoreactivity of the purified recombinant S protein. M, protein marker, lane 1, purified S protein. **D** Morphological examination of the purified S protein by transmission electron microscope. **E** Cut-off determination for PDCoV S-protein-based iELISA. Seronegative samples collected from newborn, colostrum-deprived piglets, which were confirmed to be free of PDCoV antibodies (n = 45) and seropositive samples collected post-immunization (n = 45) were enrolled. The dashed line represents the positive threshold (Mean + 3 SD) calculated from PDCoV-negative porcine sera. **F** ROC analysis of the iELISA. PDCoV-negative samples (n = 45) and PDCoV-positive samples (n = 45) were measured. **G** Specificity assay. Cross-reactivity was evaluated using positive antisera against PDCoV and eight other porcine viruses (porcine epidemic diarrhea virus, PEDV; porcine rotavirus, PoRV; transmissible gastroenteritis virus, TGEV; classical swine fever virus, CSFV; porcine reproductive and respiratory syndrome virus, PRRSV; porcine circovirus type 2, PCV2; pseudorabies virus, PRV, Getah virus, GETV). The dashed line indicates the determined cut-off value. **H** Sensitivity assay. Analytical sensitivity was determined using serial 2-fold dilutions (1:100 to 1:12,800) of PDCoV-positive serum. Data are presented as mean ± SD from three independent experiments, n = 3. **I** Correlation between iELISA-measured PDCoV IgG and NAb titers. PDCoV-negative samples (n = 18) and PDCoV-positive samples (n = 78) were measured. **J** Results of the iELISA on clinical porcine serum samples collected from three provinces.

modifications and native protein folding, while ferritin-mediated self-assembly facilitates multivalent epitope presentation (Fig. 1A). This strategic integration significantly enhanced antigen stability, providing a reliable and scalable antigen source with high batch-to-batch consistency, as the coating antigen is the key determinant of a reliable assay (Aydin et al., 2025). The iELISA demonstrated superior diagnostic performance, achieving an AUC near 1.0 and high sensitivity/specificity. The strong agreement with IFA ($\kappa > 0.9$) further confirms its accuracy (Supplementary Table S3), as does the significant correlation with NAb titers (Fig. 1I). Low intra-assay and inter-assay CV (< 8%) underscore the assay's robustness (Supplementary Table S2). Clinical application revealed a 48.64% seropositivity rate among 331 field samples from three Chinese provinces, indicating widespread and sustained viral circulation. These findings serve as an important complement for assessing the historical infection status of pig populations in the region.

Nevertheless, several limitations of the present study should be recognized. The clinical samples were restricted to specific geographical regions and a single age group, which may not fully represent national infection dynamics. As the virus continues to evolve, ongoing monitoring and testing against emerging genotypes will be essential. Such continuous validation is critical to ensure the long-term reliability and universal applicability of this diagnostic tool in the face of shifting viral epidemiology. Future research will focus on expanding sample diversity and evaluating assay performance in complex co-infection scenarios. While our iELISA IgG levels showed a strong correlation ($r = 0.95$) with neutralizing titers, this validation was limited by a relatively small cohort ($n = 40$). Consequently, expanded testing with additional post-vaccination samples is required to fully establish the assay's predictive value for vaccine efficacy (Oh et al., 2005).

In conclusion, this study provides a practical and robust serological tool for monitoring PDCoV infection and assessing vaccine-induced immunity in pig populations. Furthermore, our findings reveal the widespread prevalence of PDCoV in several regions of China, emphasizing the need for continued surveillance.

FOOTNOTES

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