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Development of a neutralizing monoclonal antibody recognizing a conserved epitope of CDV-H protein and its colloidal gold test strip for antigen detection

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ABSTRACT

Canine distemper virus (CDV) is currently a significant viral disease that poses a threat to the health of pets and the breeding of fur-bearing animals. It is crucial to develop a diagnostic method that can be performed on-site, is simple to execute, and yields intuitively visible results. In this study, a neutralizing monoclonal antibody(mAb) 5-8H targeting the CDV-H protein was developed and characterized. The mAb 5-8H specifically recognizes prevalent CDV strains. The minimal linear epitopes recognized by 5-8H were identified as epitopes 498-607aa, with key amino acids being 531D, 552R, 554T, and 599R. These four key antigenic sites exhibited a high degree of conservation among all CDV strains, with conservatism ranging from 94.39 % to 99.78 %. The three-dimensional structural revealed that the antigenic epitope was exposed on the surface of the CDV-H protein, making it accessible for binding with antibody. Based on mAb 5-8H, a highly sensitive and specific colloidal gold test strip for detecting CDV was developed. This method achieves a concordance rate of 96 % with RT-qPCR and is suitable for rapid detection of CDV.

1. Introduction

Canine distemper is an infectious disease caused by the canine distemper virus (CDV) and is associated with a high morbidity and mortality rate, with the mortality rate in infected animals exceeding 80 % [1–3].The intensification and scale of dogs and fur animals can easily lead to the epidemic outbreak of canine distemper. Unfortunately, the lack of effective on-site detection methods often results in delayed diagnosis of CDV infection, causing missed opportunities for timely intervention and significant losses to the fur animal industry [4]. Therefore, the accurate and rapid detection of CDV is of paramount importance. The CDV genome structure includes six transcription units (N-P-M-F-H-L) organized in a linear form. H protein is located on the outer layer of the CDV envelope. It is responsible for recognizing SLAM and nectin-4 receptors on the cell surface, enabling the virus to attach to the cell surface and assist CDV in fusing with host cells via its envelope to enter the host cell. Therefore, the H protein plays a crucial role in CDV cellular tropism, interspecies transmission, and mediating viral infection. Meanwhile, H protein has many neutralizing antigenic epitopes and is the main antigen to induce the body to produce neutralizing antibodies [5].

The CDV-H gene shows the highest genomic variability in the CDV spectrum. Due to the global distribution of CDV, phylogenetic genotyping of the virus is based on the CDV-H gene sequence and classifies it into at least 20 genotypes such as America-1, America-2, Asia-1, Asia-2, Africa-1, Africa-2, European wildlife, Arctic, and Europe-1/

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SouthAmerica-1 genotypes and so on [6,7]. Therefore, the development of neutralizing monoclonal antibodies (mAbs) against CDV-H protein conservation not only establishes a therapeutic foundation for the treatment of distemper, but also offers technical tools for the detection of the disease.

In this study, a neutralizing mAb was developed with CDV-H protein expressed by CHO, and the neutralizing epitope was identified and confirmed to be highly conserved. Based on this mAb and the conserved mAb previously developed in our laboratory, a method of detecting CDV by double-antibody sandwich colloidal gold strips was established, which can provide a reference method for the rapid clinical diagnosis of distemper.

2. Materials and methods

2.1. Cells, viruses and antibodies

Vero-SLAM cells, SP2/0 cells, mAb 9-7B, America-1 genotype CDV strain, Asia-4 genotype strain, and Asia-1 genotype strain were preserved by this laboratory. DMEM medium (Gibco), Roswell Park Memorial Institute (RPMI) 1640 medium, ABW serum were used. Commercially purchased HRP-coupled goat anti-mouse IgG (BF03001, Biodragon) and Alexa Fluor 488 labeled goat anti-mouse IgG (H + L) (A0428, Beyotime). Canine disease material was collected from a veterinarian.

2.2. Preparation and characterization of anti-CDV-H protein monoclonal antibodies

MAbs against CDV-H protein were prepared according to reference [8]. First, CHO system-expressed glycosylated H protein was immunized in BALB/c mouse. Blood was collected by tail-breaking for indirect ELISA to determine the antibody titer in mouse serum. When the total antibody titer reached 1:10000 and the neutralizing antibody titer reached 1:1000, cell fusion was performed. Simultaneously, indirect ELISA, indirect immunofluorescence, and neutralization—were used for screening. Positive clones were subcloned. A strain of hybridoma cells 5-8H was screened for stable secretion of mAbs. Ascitic fluid was produced and subsequently purified. The mAbs were characterized using indirect ELISA, Western blotting, indirect immunofluorescence assay (IFA), and neutralization assays. Additionally, the isotypes of the mAbs were subclassified utilizing the IsoStrip kit (Sigma-Aldrich). Non-competitive indirect ELISA was used to measure antibody affinity. The specific method involved diluting the CDV-H protein concentration to 0.03125 μg/mL using coating solution, then coating it onto a 96-well plate. At the same time, At the same time, the concentration of ascites antibody was diluted from 1600 μg/mL to 15 gradients with antibody dilution solution for indirect ELISA. The affinity curve was fitted by drawing a curve with antibody concentration as the horizontal axis and OD450 absorbance value as the vertical axis. The antibody concentration at the midpoint of the maximum value of OD450 on the curve was obtained. According to the formula K = (n-1)/2(n[Ab']t-[Ab]t), the affinity constant of the antibody to be tested could be calculated.

2.3. Western blot

The recombinant H protein samples were separated by SDS-PAGE and transferred onto the nitrocellulose (NC) membranes. The blocking solution was applied for 1 h, after which the mAb 5-8H was incubated for 1 h, followed by a 1-h incubation with HRP-conjugated goat anti-mouse antibody. Subsequently, results were observed with a chemiluminescence imaging system.

2.4. Indirect immunofluorescence experiment

Vero-SLAM cells were infected with America-1 genotype CDV strain, Asia-1 genotype CDV strain and Asia-4 genotype CDV strain in 48-cell well plates. Uninfected cells served as negative controls. After 48 h, the cells were fixed using 4 % paraformaldehyde and subsequently permeabilized with 0.02 % Triton X-100. The cells were then blocked with 10 % goat serum for 1 h at 37 °C and incubated with the mAb 5-8H for 1 h at 37 °C. For the secondary antibody, Alexa Fluor 488 labeled goat anti-mouse IgG(H + L) (A0428, Beyotime) was applied for 1 h at 37 °C. The results were observed under the fluorescence microscope (Mshot).

2.5. Neutralization test

Neutralization assays were conducted to evaluate the neutralizing activity of mAb 5-8H against the America-1, Asia-1, and Asia-4 genotype CDV strains. In brief, a twofold dilution of the mAb was incubated with 200 $TCID_{50}$ of the virus for 1 h at 37 °C. Following this, the antibody-virus mixture was introduced to Vero-SLAM cells and incubated at 37 °C. The cells were monitored every 24 h for 3 consecutive days, and cytopathic effects were recorded.

2.6. Indirect ELISA

The titer of mAb 5-8H was determined by an established indirect ELISA method. ELISA plates were coated with the recombinant CDV-H protein in buffer bicarbonate at 4 °C overnight. The plates were blocked at 37 °C for 1 h. The ascites of mAb 5-8H was diluted 10-fold vs. Then ELISA plates were incubated with the diluted ascites at 37 °C for 1 h. The supernatant of SP2/0 cells was used as negative control. Goat anti-mouse IgG-HRP was incubated at 37 °C for 1 h. The plates were incubated with substrate solution tetramethyl benzidine (TMB) at 37 °C for 10 min and the reaction was stopped with 2 M H_2SO_4 in each well. The OD450 values were read as the experimental results [9] and the clones demonstrating an OD450 ratio (tested sample/negative control) of ≥2.1 were classified as positive.

2.7. Identification of epitopes on the protein H sequence

The CDV-H protein (amino acids 59–607) was divided into five segments, and plasmids containing EGFP and HIS tags were constructed for transient expression in CHO cells. The transiently expressed truncated protein was subsequently analyzed using a Western blot (WB) reaction. The second phase of the truncated protein construction and expression was then carried out.

2.8. Sequencing and molecular docking of monoclonal antibodies

The mAb 5-8H was sequenced, and the sequencing results were submitted to NCBI for Ig Blast analysis to derive the sequences of the variable regions of both the light chain and the heavy chain of the mAb 5-8H. Subsequently, molecular docking of the identified epitopes 498-607aa with the variable region of the mAb using AlphaFold3 [10].

2.9. Biological information analysis

To investigate the homology of epitopes to CDV sequences, representative strains provided from the NCBI protein database (https://www.ncbi.nlm.nih.gov/protein/) were compared. The 446 CDV strains selected randomly contain different types were selected for comparison. The H protein phylogenetic tree was constructed based on MEGA software using the neighbor-joining method, and bootstrap values were calculated based on 1000 replicates. Conservation of key amino acids were analyzed. The three-dimensional (3D) structure of identified epitopes in H protein was analyzed by mapping epitope locations onto a 3D model using PyMOL software based on the results obtained from the SWISS-MODEL online server [11].

2.10. Establishment of double-antibody sandwich colloidal gold test strips

According to previous instructions [12], a double antibody sandwich colloidal gold immunochromatographic test strip was developed using monoclonal antibodies (mAbs) 5-8H (gold-labeled antibody) and 9-7B (capture antibody). Colloidal gold nanoparticles were synthesized via the sodium citrate reduction method and characterized by UV–Vis spectroscopy. The optimal pH for antibody conjugation was determined

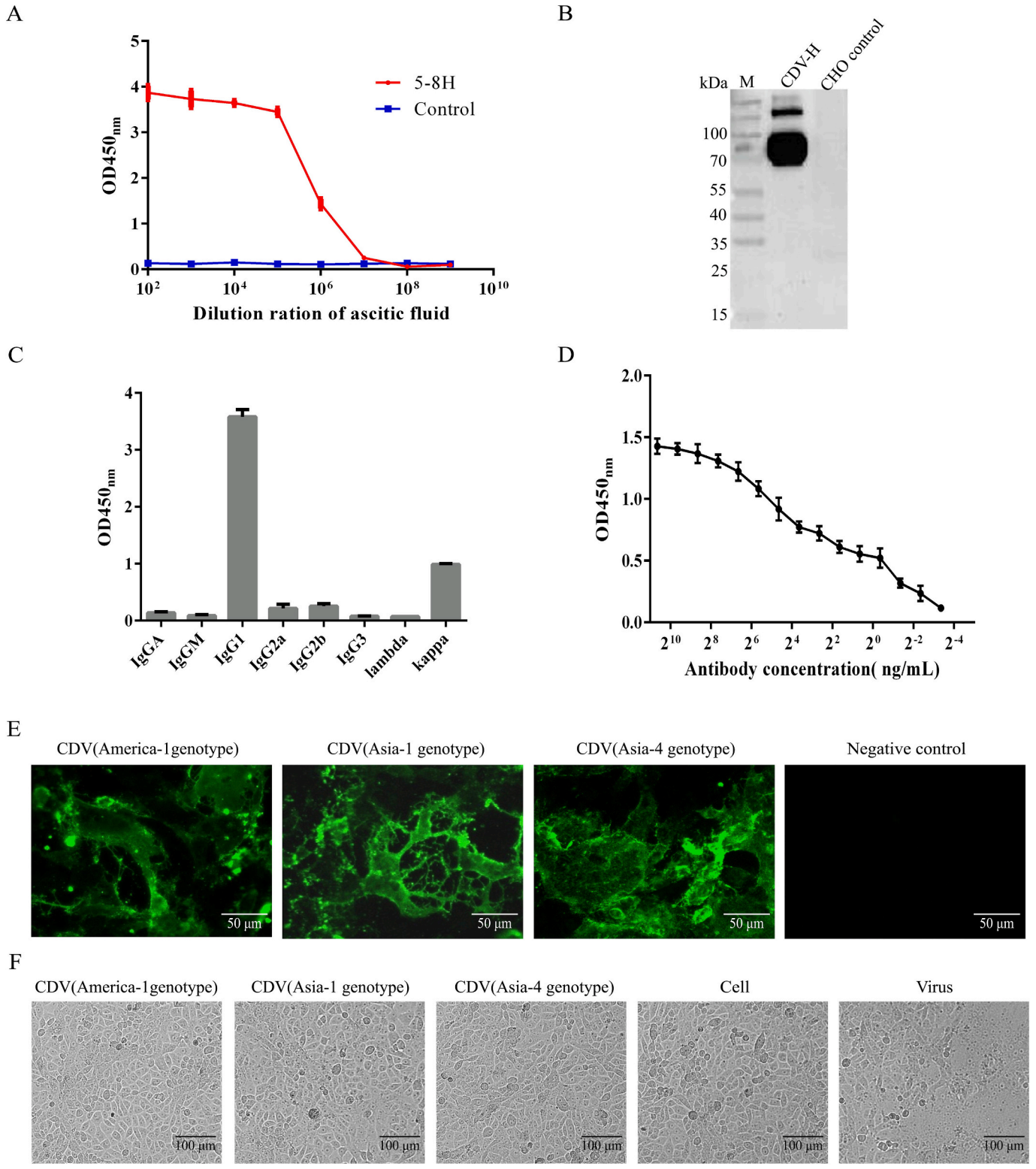


Fig. 1. Screening and characterization of mAb 5-8H. (A) Ascites titer of mAb 5-8H. Means±S.D. of data from three repeated experiments performed. (B) WB reactivity of mAb 5-8H to CDV-H. (C) Subclass identification of mAb 5-8H. Means±S.D. of data from three repeated experiments performed. (D) Determination of the affinity constant of monoclonal antibody. Means±S.D. of data from three repeated experiments performed. (E) Indirect immunofluorescence assay analysis of mAb 5-8H. (F) Neutralization assay analysis of mAb 5-8H.

by titrating 0.2 M K_2CO_3 into the colloidal gold solution. Subsequently, the minimal stabilizing antibody concentration was assessed using the Mey method. The gold-labeled mAb 5-8H, prepared under optimized conditions, was verified by UV–Vis spectrophotometry. Qualified conjugates were dispensed onto a polyester fiber membrane (200 μL/10 cm) to form the conjugate pad. The capture mAb 9-7B (2 mg/mL) and goat anti-mouse secondary antibody (1 mg/mL) were striped drawn on the nitrocellulose membrane. The test strip was assembled by sequentially laminating the sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad. Finally, strips were cut to 4 mm width and assembled into plastic cartridges for further apply.

The prepared CDV-H colloidal gold test strips were used to detect canine poliovirus (CPV), canine coronavirus (CCOV), canine adenovirus (CAV-2), canine parainfluenza virus (CPIV), and canine distemper virus (CDV) to analyze the specificity. The sensitivity of the virus solution was evaluated by detecting the viral content diluted at different multiples of CDV ($10^{5.5}$ $TCID_{50}$/mL) (Asia-1 genotype strains). The test strip was placed in 37 °C for one month to detect the same sample and observe the repeatability and stability of the test strip. Twenty-five clinically collected canine nasal or ocular secretion samples were tested with canine distemper virus H protein colloidal gold test strips, commercially available canine distemper virus N protein colloidal gold test strips (A brand with a formal national registration number) and RT-qPCR. The results of RT-qPCR were used as the determination standard. Estimate the minimum copy number of clinical positive samples detected by the test strip method.

3. Result

3.1. Development of a neutralizing mAb against CDV-H protein

In this study, an anti-CDV mAb 5-8H was successfully screened by hybridoma technique. Indirect ELISA demonstrated that 5-8H specifically recognized CDV-H protein and the antibody ascites titer reached $1{:}10^6$ (Fig. 1A, Table S1). WB results indicated that 5-8H could recognize linearized CDV-H protein (Fig. 1B). The subclass identification results indicated that the subtype of 5-8H was IgG1/Kappa type ((Fig. 1C). Antibody affinity represents the binding force between an antibody and antigenic determinants or epitopes. When the affinity constant (Ka) of an antibody is above 10^7 L/mol, it indicates that the measured antibody has good affinity. Non-competitive indirect ELISA was used to measure antibody affinity. According to the affinity calculation formula, the affinity constant (Ka) for mAb 5-8H was 1.83×10^{10} L/mol, indicating that this antibody had high affinity (Fig. 1D). To verify the reactivity and broad-spectrum of 5-8H against CDV viruses, indirect immunofluorescence experiments were performed with CDV strains kept in our laboratory, and the results indicated that 5-8H reacted specifically against three strains, which demonstrated that 5-8H had good broad-spectrum properties (Fig. 1E). The neutralization test was performed with ascites antibody. It showed that the virus group had typical cytopathic effects caused by cell fusion after CDV infection of Vero-SLAM cells, while no cytopathic effects were observed in either the cell group or the experimental group. Additionally, the neutralizing titers for America-1 genotype reached $1{:}10^2$, and those for Asia-1 genotype and Asia-4 genotype reached $1{:}10^3$. This demonstrates that 5-8H had excellent neutralizing activity (Fig. 1F).

3.2. Identification of CDV-H antigenic epitopes recognized by mAb 5-8H

The CDV-H protein (59-607aa) was truncated into 5 sections (Fig. 2A). Truncated protein was expressed by CHO expression system and the recognition effect of mAb 5-8H on truncated protein was verified by WB (Fig. 2B). The WB results indicated that only segments A1, A2, and A3 reacted specifically with mAb 5-8H (Fig. 2C). A second truncation was performed based on this result (Fig. 2D), and the results indicated that a1, a2, a3 and a4 specifically bound to mAb 5-8H (Fig. 2E and Fig. 2F). The epitope identified from these results was 498-607aa.

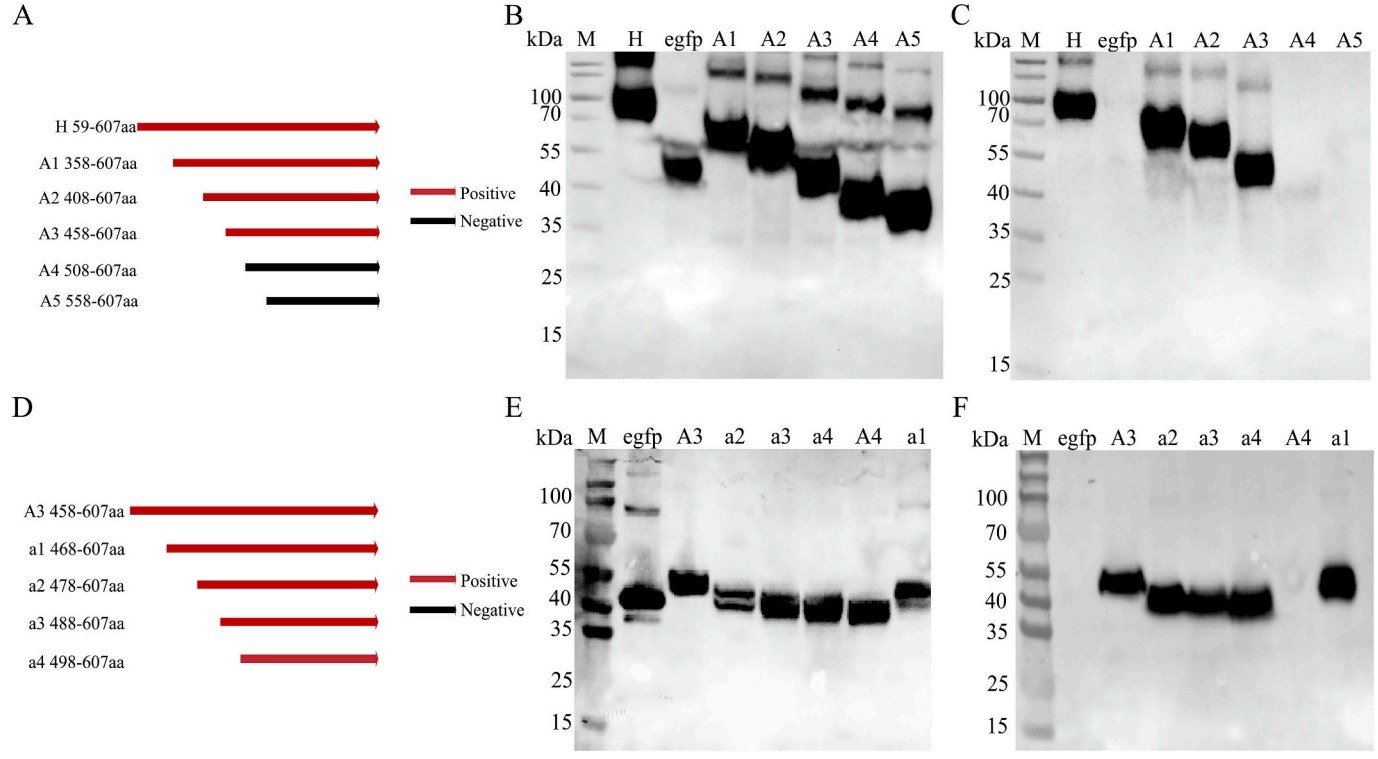


Fig. 2. Epitope identification. (A) First round of truncation. (B) WB reaction analysis of first round truncated protein with his. (C) WB reaction analysis of first round truncated protein with mAb 5-8H. (D) Second round truncation. (E) WB reaction analysis of second round truncated protein with his. (F) WB reaction analysis of the second round truncated protein with mAb 5-8H.

3.3. Localization of key amino acids

To resolve the key amino acids of the CDV-H protein recognized by mAb 5-8H. The sequences of the light and heavy chain variable regions of 5-8H were obtained by PCR amplification. Molecular docking of the epitopes 498-607aa, which were previously identified, with the variable region of the mAb 5-8H was performed using AlphaFold3. The results indicated that the predicted key amino acids were 531D, 552R, 554 T, 571D, 599R, and 606 K (Fig. 3A) (Table. S2). The alanine mutation was performed for the individual key amino acids, and the results indicated that the key amino acids were 531D, 552R, 554 T, and 599R (Fig. 3B and Fig. 3C).

3.4. Conservative analysis and localization of antigens

To assess whether the key antigenic sites were conserved in different CDV-H proteins, a phylogenetic tree of CDV-H proteins was constructed using the neighbor-joining method (Fig. 4A). These four key antigenic sites exhibited a high degree of conservation among all CDV strains, with conservatism ranging from 94.39 % to 99.78 % (Fig. 4B). PyMOL was used to visualize epitopes 498-607aa based on the crystal structure of measles virus CDV-H protein. The structure indicated that the epitope was exposed on the surface of the CDV-H protein and was susceptible to antibody binding (Fig. 4C and Fig. 4D).

3.5. Establishment of colloidal gold antigen test strip

The colloidal gold solution was successfully prepared, exhibiting a clear, transparent, and purplish-red appearance without visible aggregates or precipitation (Fig. 5A). UV–Vis spectroscopy revealed a single absorption peak at 524 nm with a bell-shaped curve, confirming high-quality colloidal gold nanoparticles (Fig. 5B). To determine the optimal pH for antibody conjugation, varying volumes of 0.2 M K_2CO_3 were titrated into the colloidal gold solution. The optimal condition was achieved with 3 μL of 0.2 M K_2CO_3 per mL of colloidal gold solution (Fig. 5C). Under this pH, mAb 5-8H was conjugated to the colloidal gold at different concentrations. The minimal stabilizing antibody concentration was found to be 10 μg per mL of colloidal gold solution (Fig. 5D). Post-optimization, the gold-labeled 5-8H antibody was characterized by UV–Vis spectroscopy, showing a distinct shift in the absorption peak to 529 nm with an increased OD value. The single peak confirmed successful antibody conjugation (Fig. 5E).

3.6. Analysis of the specificity, sensitivity, and coincidence rate of colloidal gold test strips

The specificity was evaluated using prepared CDV-H colloidal gold test strips. The results demonstrated that the test strips reacted exclusively with the CDV, indicating a high level of specificity (Fig. 6A). The sensitivity of CDV-H colloidal gold test strip was analyzed by diluting the CDV virus solution ($10^{5.5}TCID_{50}$/mL) 2 times and using the diluted

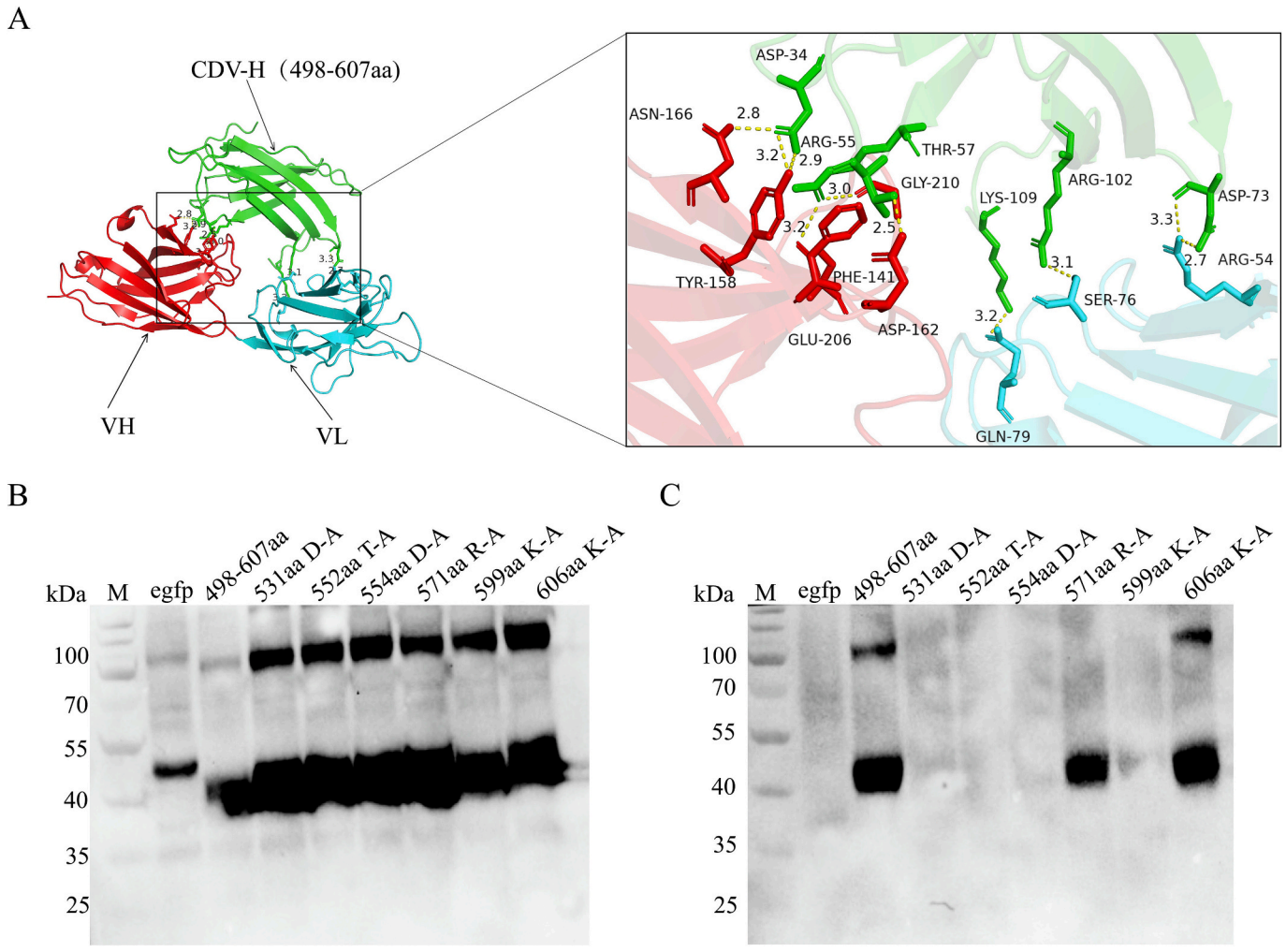
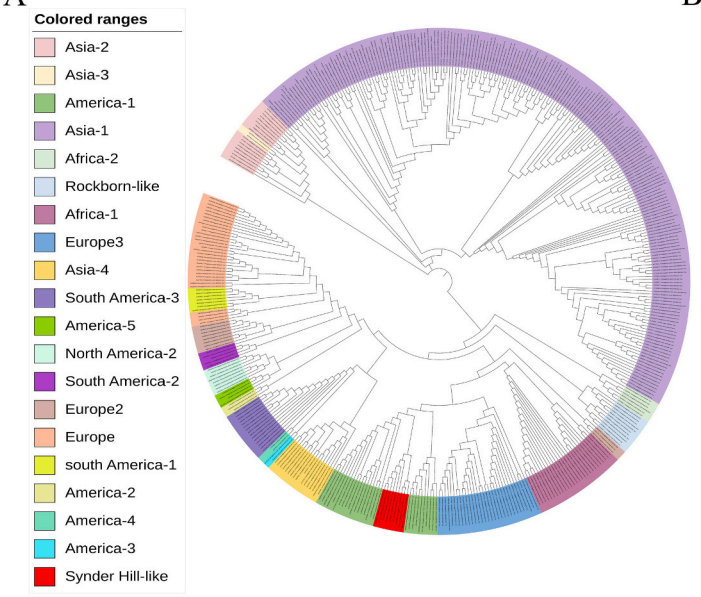


Fig. 3. Identification of key amino acids. (A) Antigen-antibody molecular docking results. Green: antigen (498-607aa); red: heavy chain; blue: light chain. The yellow dashed line is the hydrogen bond for antigen-antibody binding. (B) WB reaction of key amino acids with his. (C) WB reaction of key amino acids with mAb 5-8H.

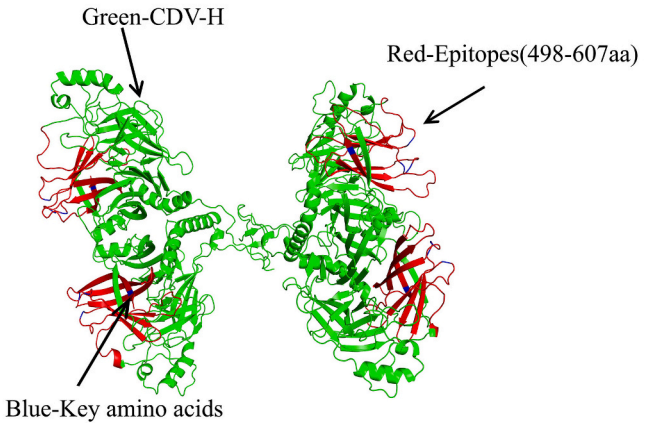
A



B

Key amino acids	Mutant site	Number	Genotype	Conservatism
531-D	N	18/18	Asia-2	94.39%
	Y	2/2	Asia-3	
	N	1/31	Europe3	
	N	3/204	Asia-1	
	N	1/2	America-3	
599-R	K	3/31	Europe	98.65%
	K	2/204	Asia-1	
	K	1/17	Asia-4	
552-R	L	1/11	Rockborn-like	99.78%
554-T	A	3/17	Asia-4	99.32%

C



D

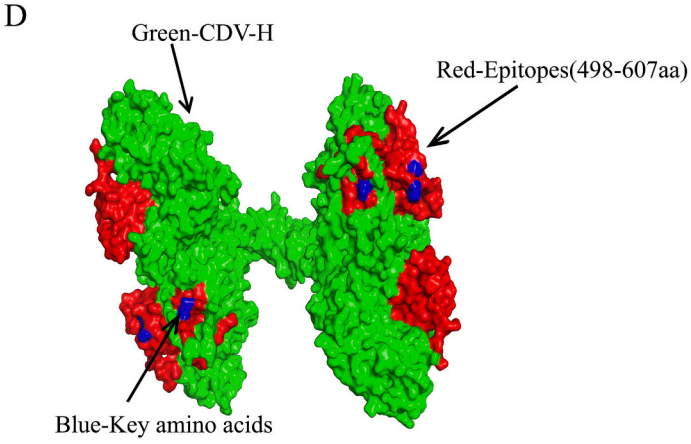


Fig. 4. Conservative analysis and localization of antigens. (A) Phylogenetic tree of 446 CDV strains based on CDV-H protein. (B) Amino acid sequence comparison of antigenic epitopes. (C) Demonstration of the tetrameric cartoon structure of CDV-H protein. (D) Display of the tetrameric surface structure of CDV-H protein.

sample for detection. The detection limit of the test strip in this study was 1:160 (CDV: $10^{3.3}TCID_{50}$/mL) confirming good sensitivity of the test strip (Fig. 6B). The test strip was placed in 37 °C for 30 days, which was equivalent to the storage at room temperature for one year. After testing, there was no significant difference between the test strip stored in 37 °C for 30 days and the first day, indicating that the stability of the test strip was good (Fig. 6C). Additionally, the CDV-H protein test strip achieved a high concordance rate of 96 % with RT-qPCR, which was higher than the 84 % concordance rate of the commercially available CDV-N protein test strip (Fig. S1) (Table. S3 and Table. 1). Therefore, the minimum detection limit of this test strip for the virus was $10^{3.3}TCID_{50}$/mL virus (Ct value of 28 or less). Therefore, the CDV-H colloidal gold test strip holds significant potential for market application.

4. Discussion

Canine distemper is a group of highly contagious and lethal infectious diseases caused by CDV, often resulting in systemic illness [13]. In recent years, the incidence of distemper has risen alongside the increasing number of pet dogs being bred, leading to a gradual expansion of damage and economic losses [14]. CDV-H envelope glycoproteins are attractive targets for vaccination or passive immunotherapy. However, H proteins are highly mutable, resulting in different CDV profiles. Because current vaccines may provide incomplete protection due to antigenic differences, it is necessary to elucidate the detailed neutralization epitopes of CDV-H proteins. The currently reported neutralizing linear epitopes are 126FNPNREFD133 [15], 193TSVGRFFPL201 [16], 238DIEREFD244 [17], and 238DIEREFDT245 [18]. In this study, we developed neutralizing mAb 5-8H against the H protein, whose binding epitopes were localized to linear epitopes 498-607aa, with key amino acids were 531D, 552R, 554 T, and 599R. These key amino acids spanned a wide range, indicating that although the mAb 5-8H recognized a linear epitope, it was shown by the position of the key amino acids that the mAb 5-8H still recognized a spatial conformation of the epitope. At the same time, the antigenic epitopes and key amino acids have not been reported. Analyzing the conservatism of the key amino acids, the four key antigenic sites showed a high degree of conservatism of 94.39 %–99.78 % among all CDV strains. This not only provides precise antigenic site information for vaccine design, but also proves that the antibody 5-8H developed in this study has a broad-spectrum recognition effect. Meanwhile, structural modeling showed that the linear epitope recognized by mAb 5-8H was exposed to H protein indicating that the epitope had good antigenicity.

Canine distemper virus colloidal gold test strips currently represent the most widely adopted detection method for clinical diagnosis. However, conventional whole-virus based test strips demonstrate suboptimal sensitivity and specificity. While nucleocapsid (N) protein-targeting test strips offer improved performance, they present notable limitations:

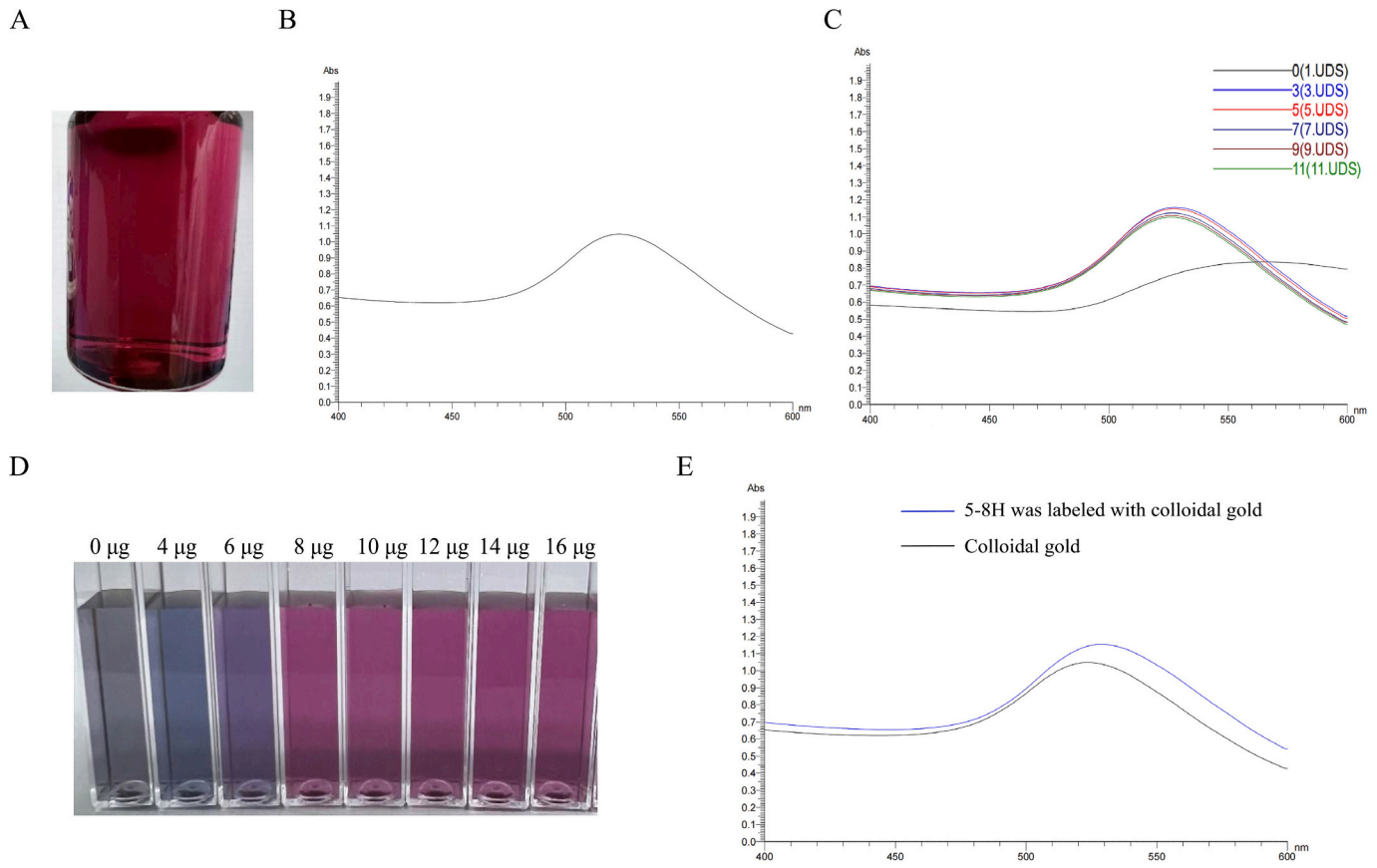


Fig. 5. Development of the colloidal gold-based immunochromatographic test strip. (A) Colloidal gold nanoparticles were identified by the naked eye. (B) UV–Vis spectroscopy (400–600 nm) of colloidal gold nanoparticles, displaying a characteristic peak at 524 nm. (C) pH optimization for colloidal gold conjugation. The absorption peak intensity decreased progressively with increasing volumes of 0.2 M K_2CO_3 added per mL of colloidal gold solution. (D) Optimization of monoclonal antibody (5-8H) concentration for colloidal gold labeling. At 6 μg/mL, the solution exhibited purple discoloration and precipitation (insufficient stabilization), while 10 μg/mL maintained stable red dispersion (optimal stabilization). (E) UV–visible spectrum verification of successfully labeled 5-8H-gold conjugates, showing a shifted peak (529 nm) and increased absorbance.

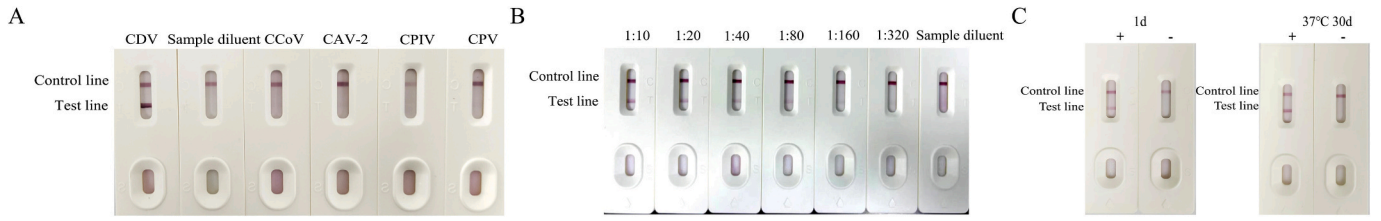


Fig. 6. Specificity and sensitivity analysis of test strips. (A) Specificity analysis of test strips. (B) Sensitivity analysis of test strips. (C) Stability detection of test strips.

Table 1
Coincidence rate analysis of test strips.

Assay	Positive	Negative	Total	Coincidence rate
CDV-H colloidal gold test strip	5	20	25	96 %
CDV-N colloidal gold test strip	2	23	25	84 %
RT-qPCR	6	19	25	/

potential cross-reactivity with N proteins from other *Paramyxoviridae* family members may yield false-positive results, and excessive proteolytic cleavage of the N protein can compromise antibody binding efficiency, further contributing to diagnostic inaccuracies. These technical challenges highlight the need for more reliable detection alternatives in clinical settings [19,20]. To address these issues, the development of highly sensitive and highly conserved mAbs is particularly critical [21]. The antigen recognized by the mAb developed in this study is highly conserved. The double antibody sandwich colloidal gold strip detection method was established based on this conserved mAb and another mAb targeting a conserved spatial epitope identified in a previous study. The detection system developed in this study exhibited exceptional specificity, showing positive reactions exclusively with CDV-infected samples while demonstrating no cross-reactivity with other prevalent canine viral pathogens. Comparative analysis of clinical samples revealed superior diagnostic performance of the CDV-H colloidal gold test strips, which achieved a 96 % concordance rate with RT-qPCR results and outperformed conventional CDV-N test strips in detection sensitivity. The minimum detection limit of this test strip for the virus was $10^{3.3}TCID_{50}$/mL virus (Ct value of 28 or less), confirming its sensitivity for clinical applications.

Comparative evaluation of the three detection methods revealed that while the commercial CDV-N colloidal gold test strip achieved detection in clinical samples with CT values up to 20, the CDV-H test strip

developed in this study demonstrated superior sensitivity with detectable signals at CT values as high as 28. This enhanced performance likely stems from fundamental differences in target protein biology and detection mechanisms. The N protein, despite its high viral copy number, requires proteolytic cleavage for immunodetection, and commercial test strips may employ conservative cleavage conditions to prevent false positives, potentially limiting N protein release in samples with low viral loads (high CT values). In contrast, our antibodies target highly conserved epitopes on the native H protein, enabling direct detection of intact virions without cleavage-dependent antigen release. Furthermore, the H protein maintains stable conformational epitopes even at low concentrations, facilitating reliable antibody recognition. These combined advantages of H protein targeting, including elimination of cleavage-dependent antigen loss and preservation of native protein conformation at low viral loads, account for the significantly improved detection sensitivity of our colloidal gold test strip compared to conventional N protein-based assays.

The CDV-H colloidal gold test strip developed in this study establishes a solid theoretical foundation and provides a reliable detection method for clinical applications. While this H protein-based test strip overcomes several limitations associated with whole-virus and N protein-based colloidal gold assays, certain constraints remain noteworthy. Specifically, the relatively lower expression levels of H protein compared to N protein may potentially result in false-negative outcomes during early-stage infections. Additionally, transient positive results could occur following administration of attenuated virus vaccines or H protein subunit vaccines. To optimize diagnostic accuracy, we recommend combining early-stage detection with CDV-N colloidal gold test strips and carefully considering vaccination history during test interpretation. These considerations highlight the importance of context-specific application while demonstrating the significant advantages of our CDV-H detection system for clinical use.

In conclusion, this study developed a novel neutralizing mAb 5-8H against the CDV-H protein. The recognition epitopes of 5-8H are located within amino acids 498–607, with key amino acids 531D, 552R, 554 T, and 599R exhibiting highly conserved, which enriched the understanding of the immune epitopes present in CDV-H protein. Additionally, a colloidal gold test strip detection method for detecting CDV was successfully developed. The established detection method has high coincidence rate, high sensitivity, and good specificity, which provides a reference method for rapid clinical diagnosis of CDV.

Ethics approvals

All animal experiments in this study followed the ethical guidelines for animal experiments of the Laboratory Animal Center of Huazhong Agricultural University and were approved by the Animal Ethics Committee of the Laboratory Animal Center of Huazhong Agricultural University (approval number:HZAUMO-2025-0018).

CRediT authorship contribution statement

Zhengguo Wang: Supervision, Methodology, Conceptualization, Writing – original draft. **Linlin Mu:** Methodology, Investigation, Data curation. **Guishan Ye:** Methodology. **Kuijing He:** Methodology. **Cong Cai:** Methodology. **Peiyao Shi:** Investigation. **Jieyu Zhou:** Investigation. **Yaqin Gan:** Investigation. **Yue Zeng:** Investigation. **Anding Zhang:** Methodology, Data curation, Conceptualization. **Long Li:** Methodology, Funding acquisition, Writing – review & editing. **Li Han:** Funding acquisition, Data curation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Statement

During the preparation of this work, the authors used Ernie Bot in order to improve the readability and language of the manuscript. After using Ernie Bot, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2025.144800.

Data availability

The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

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