

A capsid protein-based indirect ELISA for detection of goose astrovirus antibodies

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Abstract

Goose astrovirus (GAstV) is a major pathogen associated with gout in goslings. Currently, no effective therapeutics or vaccines are available against GAstV infection, and disease control primarily relies on strict biosecurity measures. Therefore, the development of rapid and reliable diagnostic methods is critical for clinical management and reducing the impact of the virus. In this study, an indirect enzyme-linked immunosorbent assay (ELISA) for the detection of GAstV was developed. The N-terminal region (amino acids 130–401) of the GAstV capsid protein was cloned into the prokaryotic expression vector pET-32a(+) and expressed in *Escherichia coli* BL21 cells. The recombinant protein, designated GAstV-Cap-N, was purified and used as the coating antigen in the ELISA. Following systematic optimization of key parameters, the established indirect ELISA exhibited high specificity, sensitivity, and reproducibility. Comparative analysis with Western blot demonstrated a concordance rate of 87.5%. The assay was subsequently applied to test 228 clinical serum samples from geese, revealing an overall seropositivity rate of 38.19%. These findings indicate that the developed indirect ELISA provides a robust and reliable tool for clinical diagnosis and epidemiological surveillance of GAstV.

Introduction

Goose astrovirus (GAstV) belongs to the family *Astroviridae*, genus *Avastrovirus* (Zhu & Sun, 2022). It is a small, non-enveloped virus with a non-segmented, single-stranded, positive-sense RNA genome of approximately 7.1 to 7.3 kilobases. The viral genome consists of 5' and 3' untranslated regions (UTRs), three open reading frames (ORF1a, ORF1b, and ORF2), and a poly(A) tail (M. Li et al., 2025). Among these, ORF1a spans approximately 3,255 nucleotides (nt) and encodes a non-structural protein of 1,085 amino acids (aa). ORF1b, approximately 1,551 nt in length, encodes the RNA-dependent RNA polymerase. ORF2 comprises 2,115 nt and encodes the capsid protein, which consists of approximately 705 aa (W. Li et al., 2025). The capsid protein serves as the primary immunogenic protein of GAstV and is capable of inducing neutralizing antibodies. It contains a highly conserved N-terminal region and a highly variable C-terminal spike domain (Ykema & Tao, 2021).

GAstV is a newly identified astrovirus that was first discovered in China in 2017. It currently comprises two genotypes (GAstV-1 and GAstV-2), with genotype GAstV-2 being the predominant circulating strain (Wang et al., 2023). GAstV primarily causes gout, renal swelling, hemorrhage, and high mortality in goslings aged 4 to 16 days (Xu et al., 2024; Zhu et al., 2022). The virus is widely prevalent in China and has caused substantial economic losses to the goose industry. At present, no effective drugs or vaccines are available against this virus. Therefore, routine surveillance of the virus in farming operations is of critical importance for its clinical prevention and control.

In previous work, our laboratory isolated two GAstV-2 strains (GXNN and GDCS) from goose farms in Guangxi and completed their whole-genome sequencing and pathogenicity analysis (Xu et al., 2024). To further establish an indirect ELISA detection method for this virus, the present study amplified the truncated N-terminal region of the capsid protein from the GXNN strain, cloned it into the prokaryotic expression vector pET-32a(+), and induced recombinant protein expression. Subsequently, the purified GAstV-Cap-N protein was used as a coating antigen to establish an indirect ELISA for the detection of GAstV. The findings of this study are expected to provide a foundation for clinical monitoring and the prevention and control of GAstV infections.

Materials and methods

Viruses, plasmids, sera, and antibodies

The GAstV GXNN strain was isolated in our laboratory in 2023 from goslings exhibiting symptoms of gout (Xu et al., 2024). The prokaryotic expression vector pET-32a(+) is maintained in our laboratory. GAstV-positive and GAstV-negative serum samples were previously identified and are also stored in our laboratory. HRP-labeled rabbit anti-goose IgY antibody was commercially obtained from Frdbio Bioscience & Technology Co., Ltd. (Wuhan, China).

Amplification of the target gene and construction of the recombinant plasmid

Specific primers were designed based on the capsid protein gene sequence of the GAstV GXNN strain (GenBank accession no. PP444709) to amplify a truncated N-terminal capsid gene segment (encoding amino acids 130 to 401). The forward primer (GAstV-Cap-F: 5'-ATCGGATCCATGCTTGCTCCCACTTGCTGG-3') contains a *Bam*HI restriction site, while the reverse primer (GAstV-Cap-R: 5'-GTGCTCGAGTCACATTTGGCTGTGTTAGC-3') contains an *Xho*I restriction site. Total RNA was extracted from GAstV using an RNA extraction kit (Tiangen, Beijing, China), and full-length cDNA was synthesized using a reverse transcription kit (TaKaRa, Dalian, China). The truncated capsid gene was subsequently amplified via PCR using the specific primer pair GAstV-Cap-F and GAstV-Cap-R. The PCR thermal profile consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 35 s, with a final extension at 72°C for 5 min.

Both the amplified target gene and the prokaryotic expression vector pET-32a(+) were digested with the restriction endonucleases *Bam*HI and *Xho*I (TaKaRa). The digested insert was then ligated into the digested pET-32a(+) vector using T4 DNA ligase to construct the recombinant plasmid pET-32a-GAstV-Cap-N, which was subsequently confirmed by sequencing (Sangon Biotech Co., Ltd.).

Expression and purification of the recombinant protein

The recombinant plasmid pET-32a-GAstV-Cap-N was transformed into chemically competent *E. coli* BL21(DE3) cells (TaKaRa). A single colony was selected and cultured with constant shaking at 37°C and 200 rpm. When the optical density at 600 nm (OD₆₀₀) reached 0.4–0.6, isopropyl β-D-1-thiogalactopyranoside (IPTG, Solarbio, Beijing) was added to a final concentration of 0.5 mmol/L to induce protein expression, and the culture was incubated for an additional 12 h. The bacterial cells were then collected by centrifugation, resuspended in PBS, and disrupted by ultrasonication. The supernatant and insoluble fraction were separated by centrifugation. The pellet was resuspended in PBS, denatured by adding 5× loading buffer, and analyzed by SDS-PAGE to verify expression of the recombinant GAstV-Cap-N protein.

For protein purification, the pellet obtained after sonication was solubilized in 8 M binding buffer. The recombinant protein was purified using nickel-agarose resin (Yeasten Biotechnology, Shanghai, China) according to the manufacturer's instructions. Briefly, a chromatography column was packed with the resin, and the protein solution was loaded onto the column. The mixture was gently agitated on a shaker at 4°C for 2 h to allow binding. The column was then washed with binding buffer to remove non-specifically bound proteins, and the target protein was eluted with elution buffer. The flow rate was maintained at approximately 1 mL/min throughout the procedure. The purified protein was mixed with 5× loading buffer, denatured at 100°C for 10 min, and analyzed by SDS-PAGE. Subsequently, the protein was transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% (w/v) skim milk for 1 h, incubated with a His-tag mouse monoclonal antibody (Proteintech, Wuhan) at 4°C for 12 h, and then probed with an HRP-labeled goat anti-mouse IgY secondary antibody at room temperature for 1 h. Protein expression was finally visualized using an enhanced chemiluminescence (ECL) detection system.

Screening of GAstV-negative and -positive sera

To identify GAstV-negative and -positive sera, purified GAstV-Cap-N protein was used as the coating antigen. Clinical goose serum samples were analyzed using a standard indirect ELISA protocol. Serum samples with the lowest and highest OD₄₅₀ nm values (six samples each) were selected as negative and positive reference sera, respectively. Subsequently, the purified GAstV-Cap-N protein served as the detection antigen. Following the same procedure as described above, the samples were subjected to SDS-PAGE and transferred onto a PVDF membrane. Western blot analysis was then performed using the presumptive negative and positive sera as primary antibodies, and HRP-labeled rabbit anti-goose IgY antibody as the secondary antibody, to confirm the expression of the GAstV capsid protein.

Determination of optimal antigen coating concentration and serum dilution

Purified GAstV-Cap-N protein was coated onto 96-well plates at concentrations of 50, 100, 200, and 400 ng per well, followed by overnight incubation at 4°C. After washing three times with PBST, the plates were blocked with 5% skim milk at 37°C for 2 h and then washed again three times with PBST. Positive and negative sera were diluted at ratios of 1:100, 1:200, 1:400, and 1:800. Subsequently, 100 µL of each dilution was added per well, and the plates were incubated at 37°C for 2 h. A 1:5000 dilution of HRP-labeled rabbit anti-goose IgY antibody was then added and incubated at 37°C for 1 h. The reaction was developed using TMB substrate (Solarbio) at room temperature for 20 min, terminated with stop solution (Solarbio), and the OD₄₅₀ nm values were measured. The optimal antigen coating concentration and serum dilution were determined by calculating the positive-to-negative serum (P/N) ratio.

Optimization of antigen coating conditions

To establish the optimal antigen coating conditions, the indirect ELISA procedure described above was repeated with modifications to the incubation parameters. The coated plates were incubated either overnight at 4°C or at 37°C for 4 h, 2 h, or 1 h. The OD₄₅₀ nm values were recorded for each well, and the P/N ratios were calculated under each condition to identify the most favorable coating protocol.

Determination of optimal primary antibody incubation time

Using the optimized conditions established above, the primary antibody incubation time was evaluated at 4 h, 2 h, and 1 h. The OD₄₅₀ nm values were measured for each well, and the corresponding P/N ratios were calculated to determine the optimal incubation duration for the primary antibody.

Optimization of enzyme-labeled secondary antibody dilution and incubation time

Under the previously optimized assay conditions, the HRP-labeled rabbit anti-goose IgY antibody was diluted at ratios of 1:5000, 1:10000, 1:20000, and 1:40000, and incubated for 30, 45, or 60 minutes. The OD₄₅₀ nm values were measured for each combination, and the P/N ratios were calculated to determine the optimal dilution and incubation time for the enzyme-conjugated secondary antibody.

Optimization of blocking buffer and incubation time

To establish the optimal blocking conditions, the indirect ELISA procedure was performed using different blocking buffers—specifically, 1% and 5% BSA, and 1% and 5% skim milk—with incubation times of 60 and 120 minutes. The OD₄₅₀ values were recorded for each condition, and the corresponding P/N ratios were calculated to identify the most effective blocking protocol.

Determination of optimal color development duration

Following the optimized conditions outlined above, the indirect ELISA was conducted with color development times of 15, 20, 25, and 30 minutes. The OD₄₅₀ nm values were measured for each time point, and the P/N ratios were calculated to determine the optimal duration for the chromogenic reaction.

Establishment of the cut-off value

Using the optimized indirect ELISA protocol, 24 known negative serum samples were tested, with each sample measured in triplicate. The mean (X) and standard deviation (SD) of the OD₄₅₀ nm values were calculated for each sample. Samples with

OD₄₅₀ nm values $\geq X + 3$ SD were classified as positive; those with OD₄₅₀ nm values $\leq X + 2$ SD were considered negative; and samples exhibiting OD₄₅₀ nm values between $X + 2$ SD and $X + 3$ SD were designated as equivocal.

Specificity, sensitivity, reproducibility, and concordance with Western blot analysis of the indirect ELISA

To evaluate the specificity of the established indirect ELISA, positive serum samples against GAstV, TMUV, NDV, and reovirus were analyzed under the optimized conditions. For sensitivity assessment, three GAstV-positive serum samples were serially diluted at ratios of 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200, and tested using the optimized indirect ELISA.

Reproducibility was examined by performing intra- and inter-assay analyses. GAstV-positive and negative serum samples were tested in triplicate across three separate plates, and the OD₄₅₀ nm values were recorded to evaluate both within-plate and between-plate consistency.

To validate the reliability of the indirect ELISA results, LMH cells were infected with the GAstV-GXNN strain at an MOI of 0.1. At 24 hours post-infection, total proteins were collected, separated by SDS-PAGE, and transferred onto a PVDF membrane. The membrane was blocked with 5% BSA for 1 h, washed three times with TBST, and then probed with three GAstV-positive and five negative sera (identified by indirect ELISA) as primary antibodies, followed by an HRP-labeled rabbit anti-goose IgY secondary antibody. Expression of the GAstV capsid protein was detected by Western blotting, and the concordance rate between the indirect ELISA and Western blotting results was calculated.

Clinical application of the indirect ELISA

To investigate the seroprevalence of GAstV antibodies in goose populations, 288 serum samples were collected from farms in Nanning and Hechi, Guangxi. The established indirect ELISA was used to detect GAstV antibodies, and the positive rate was subsequently determined.

Ethics statement

All clinical goose serum samples were collected from local farms during routine disease surveillance and diagnostic testing. Sample collection was performed by licensed veterinarians following standard management practices, and no additional invasive procedures were applied to the animals solely for this study. The use of these residual diagnostic samples was approved by the Guangxi University Animal Care & Welfare Committee (Protocol No. GXU2024–175).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 6.0 software. Intergroup differences were evaluated by one-way analysis of variance (ANOVA). Data are presented as mean $\pm$ standard deviation, with significance denoted as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Results

Truncated expression and purification of the capsid protein

In this study, the N-terminal region (amino acids 130–401) of the GAstV capsid protein was amplified by RT-PCR, cloned into the prokaryotic expression vector pET-32a(+), and used to construct the recombinant plasmid pET-32a-GAstV-Cap-N. This plasmid was then transformed into *E. coli* strain BL21(DE3). Following induction with IPTG, the recombinant protein was expressed and purified using nickel-agarose resin. The expected molecular weight of the recombinant protein GAstV-Cap-N was 49 kDa, and it was predominantly expressed as inclusion bodies (Fig. 1).

Determination of optimal antigen coating concentration and serum dilution

Using a checkerboard titration approach, the GAstV-Cap-N protein was coated onto 96-well plates at graded concentrations of 50, 100, 200 and 400 ng per well. GAstV-positive and negative sera were diluted at ratios of 1:100, 1:200, 1:400 and 1:800, and the OD₄₅₀ nm values were measured. The highest P/N ratio of 6.41 was achieved with a coating concentration of 100 ng per well and a serum dilution of 1:100 (Table 1).

Table 1
Determination of the optimal coating concentration for GAstV-Cap-N and the optimal serum dilution for indirect ELISAs.

Antigen coating concentration (ng/well)	Positive (P) or Negative (N) serum	Serum dilution			
		1:100	1:200	1:500	1:1000
50	P	0.5835	0.4727	0.295	0.168563
	N	0.0954	0.0928	0.0862	0.0842
	P/N	6.12	5.09	3.42	2.00
100	P	0.7522	0.5554	0.3109	0.2423
	N	0.1174	0.107	0.1024	0.0958
	P/N	6.41	5.19	3.04	2.53
200	P	0.8209	0.6268	0.3577	0.2455
	N	0.1305	0.1157	0.1116	0.1056
	P/N	6.29	5.42	3.21	2.32
400	P	0.8291	0.6106	0.3547	0.2571
	N	0.1526	0.1206	0.1164	0.111
	P/N	5.43	5.06	3.05	2.32

Optimization of antigen coating conditions

Based on the optimized antigen coating concentration and serum dilution, indirect ELISA was performed under varying coating conditions: overnight at 4°C, or incubation at 37°C for 1, 2, 4, and 12 h. The OD₄₅₀ nm value for each well was measured. Coating the GAstV-Cap-N protein at 37°C for 1 h yielded the highest P/N ratio of 3.64 (Table 2).

Table 2
Determination of the optimal antigen coating conditions for indirect ELISAs.

Antigen coating temperature	Antigen coating time	Positive (P) or Negative (N) serum		
		P	N	P/N
4°C	12 h	2.354575	0.869075	2.709289
37°C	4 h	2.360775	0.949825	2.485484
37°C	2 h	2.284775	0.8372	2.729067
37°C	1 h	2.406375	0.6603	3.644366

Determination of optimal sera incubation time

To establish the optimal sera incubation time, the previously optimized conditions were employed with incubation periods set at 60, 90, and 120 minutes. The OD₄₅₀ nm values were measured, and the corresponding P/N ratios were calculated. The highest P/N ratio of 3.88 was obtained when the primary antibody was incubated for 90 minutes (Table 3).

Table 3
Determination of the optimal serum incubation time for indirect ELISA

Serum incubation time	Positive (P) or negative (N) serum	
60 min	P	0.403333
	N	0.1079
	P/N	3.738029
90 min	P	0.3891
	N	0.1003
	P/N	3.879362
120 min	P	0.336633
	N	0.0986
	P/N	3.414131

Optimization of enzyme-labeled secondary antibody dilution and incubation time

To determine the optimal dilution and incubation time for the enzyme-labeled secondary antibody, dilutions of 1:5000, 1:10000, 1:20000, and 1:40000 were tested in combination with incubation times of 30, 45, and 60 minutes. The OD₄₅₀ nm values were recorded for each condition. The highest P/N ratio of 5.16 was achieved when the secondary antibody was diluted at 1:20,000 and incubated for 30 minutes (Table 4).

Table 4
Determination of the optimal dilution and incubation time of the enzyme-labeled secondary antibody for indirect ELISA

Serum dilution	Positive (P) or Negative (N) serum	Serum incubation time		
		30min	45min	60min
1:5000	P	1.937067	2.225333	2.200333
	N	0.6592	1.0975	1.3193
	P/N	2.938511	2.027639	1.667804
1:10000	P	1.735467	1.923267	2.067767
	N	0.417367	0.626367	0.925133
	P/N	4.158134	3.070513	2.235101
1:20000	P	1.312467	1.702067	1.8229
	N	0.254233	0.448733	0.6208
	P/N	5.16245	3.793047	2.936372
1:40000	P	0.8316	1.276767	1.359567
	N	0.172867	0.311733	0.395933
	P/N	4.810644	4.095701	3.433827

Determination of optimal blocking buffer and blocking time

Using the previously optimized conditions, the blocking step in the indirect ELISA was performed with 1% and 5% BSA, as well as 1% and 5% skim milk, as blocking buffers. Incubation was carried out at 37°C for either 60 or 120 minutes. The results indicated that 1% BSA was the optimal blocking buffer, with the highest P/N ratio of 4.19 achieved when blocking was conducted at 37°C for 1 hour (Table 5).

Table 5
Determination of the optimal blocking buffer and blocking time
for indirect ELISA

Blocking buffer	Positive (P) or Negative (N) serum	Blocking time	
		60 min	120 min
1% BSA	P	0.613067	0.620767
	N	0.146467	0.148933
	P/N	4.185708	4.168084
5% BSA	P	0.617333	0.633633
	N	0.1795	0.212967
	P/N	3.439183	2.97527
1% skimmed milk	P	0.596867	0.563867
	N	0.198767	0.154567
	P/N	3.002851	3.648048
5% skimmed milk	P	0.457533	0.4693
	N	0.171533	0.164867
	P/N	2.667314	2.846543

Determination of optimal color development time

To establish the optimal color development time for the indirect ELISA, incubation periods of 15, 20, 25, and 30 minutes were evaluated. The OD₄₅₀ nm values were measured under each condition. The results demonstrated that a development time of 15 minutes yielded the highest P/N ratio of 4.76 (Table 6).

Table 6
Determination of the optimal color development time for indirect ELISA

Color development time	Positive (P) or Negative (N) serum	
15 min	P	1.614467
	N	0.339367
	P/N	4.757293
20 min	P	0.794
	N	0.422567
	P/N	4.245484
25 min	P	1.779067
	N	0.473667
	P/N	3.755947
30 min	P	1.9803
	N	0.613767
	P/N	3.22647

Establishment of the cut-off value

To establish the cut-off value for the GAsTV capsid protein-based indirect ELISA, 24 GAsTV-negative serum samples were analyzed. The mean OD_{450 nm} value was determined to be 0.360, with a standard deviation of 0.078. The individual OD_{450 nm} values for each sample are presented in Table 7. A sample was classified as positive if its OD_{450 nm} value was ≥ 0.595 , negative if ≤ 0.517 , and equivocal if the value fell between 0.517 and 0.595.

Table 7
Determination of the cut-off value for indirect ELISA

Sample number	OD _{450 nm}							
1–8	0.4385	0.1274	0.3562	0.3794	0.4238	0.3723	0.3174	0.4038
9–16	0.4141	0.366	0.3548	0.4614	0.3657	0.1756	0.3479	0.34
17–24	0.4117	0.3206	0.3331	0.4018	0.4514	0.386	0.2839	0.4135
Average value ($\bar{x}$)	0.3603							
Standard deviation (SD)	0.0783							
$\bar{x}+2SD$	0.5168							
$\bar{x}+3SD$	0.5949							

Specificity, sensitivity, reproducibility, and concordance with Western blot analysis of the indirect ELISA

The GAsTV capsid protein-based indirect ELISA established in this study was used to detect antibodies against GAsTV, TMUV, NDV, and reovirus in corresponding positive sera. The results showed that only the GAsTV-positive serum tested positive, whereas all other sera were negative (Table 8), indicating high specificity of the assay for GAsTV antibody detection. Subsequently, GAsTV-positive serum was serially diluted to 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, and

1:6400, and tested using the indirect ELISA. Even at a dilution of 1:6400, the serum sample remained positive (Fig. 2), demonstrating the excellent sensitivity of the newly developed assay. To evaluate reproducibility, GAstV-positive and negative serum samples were tested in triplicate across different batches of 96-well plates using the optimized indirect ELISA protocol. The intra-assay and inter-assay coefficients of variation (CVs) ranged from 0.6% to 4.7% and 1.1% to 8.2%, respectively, both below 10%, indicating high reproducibility of the method (Table 9).

Table 8
Detection of different virus-positive sera by indirect ELISA

Serum sample	OD _{450 nm}	Detection result
TMUV	0.0549	N
NDV	0.0608	N
Reovirus	0.0596	N
ddH2O	0.049	Negative control
GAstV	1.8697	Positive control

Table 9
Reproducibility of the detection results for indirect ELISA

Negative or positive serum	number	Intra-plate duplication				Inter-plate duplication			
		1	2	3	CV	1	2	3	CV
Positive Serum	1	1.8697	1.8719	1.8377	0.010293	1.8524	1.822333	1.8143	0.010977
	2	1.9064	1.837	1.8331	0.022186	1.85465	1.789633	1.8526	0.020171
	3	1.7805	1.8372	1.887	0.029041	1.871675	1.7918	1.8489	0.022394
	4	1.853	1.8725	1.8526	0.006118	1.8725	1.7955	1.8433	0.02116
Negative Serum	1	0.5736	0.5552	0.5452	0.025816	0.4656	0.5415	0.535733	0.082163
	2	0.533	0.5431	0.5442	0.01143	0.5188	0.4923	0.490767	0.031483
	3	0.5687	0.5225	0.5665	0.047165	0.551967	0.564225	0.516533	0.045506
	4	0.5816	0.5452	0.552	0.034585	0.5442	0.5326	0.5186	0.024104

To assess the accuracy and reliability of the established indirect ELISA, eight clinical goose serum samples were analyzed in parallel by both indirect ELISA and Western blot. Indirect ELISA identified 3 positive and 5 negative samples, while Western blot detected 2 positive and 6 negative samples (Fig. 3). The concordance rate between the two methods was 87.5%.

Detection of GAstV antibodies in goose populations

A total of 288 clinical goose serum samples were collected from Nanning and Hechi in Guangxi and tested for GAstV antibodies using the indirect ELISA established in this study. The overall seropositivity rate was 38.19% (110/288). Specifically, the positive rates in Nanning and Hechi were 52.25% (58/111) and 29.38% (52/177), respectively. These findings indicate that GAstV is prevalent in goose populations in these two major cities of Guangxi.

Discussion

Since its discovery in 2016, GAsV has been detected in various regions of China (Chen et al., 2024; Fei et al., 2022; Zhang et al., 2022); however, in-depth research on its epidemiology, transmission routes, and pathogenicity remains limited. The GAsV GXNN strain used in this study represents the first goose astrovirus isolated from goose flocks in Guangxi, China (Xu et al., 2024). Currently, epidemiological investigations on GAsV are scarce. Primary detection methods for GAsV mainly rely on RT-PCR or quantitative PCR (Li et al., 2023; Yi et al., 2022). Although these techniques offer high sensitivity and specificity, they require viral nucleic acid extraction and reverse transcription, making the process time-consuming and costly. Moreover, they can only identify pathogens present during active infection. In contrast, the indirect ELISA developed in this study detects antibody levels, providing a cost-effective, high-throughput approach to assess the infection and immune status of goose populations, thereby enhancing our understanding of the virus's prevalence in geese. The advantages of indirect ELISA—economic feasibility, scalability, and operational simplicity—make it particularly suitable for clinical serological surveys (Alibeiki et al., 2020).

The product encoded by the ORF2 gene of astrovirus is the major structural protein, also known as the capsid protein. This protein plays a pivotal role in viral entry into host cells and mediates proteolytic maturation, directly influencing viral pathogenicity. Furthermore, it exhibits strong immunogenicity and reactivity, participates in the recognition of cell-surface receptors, and contributes to the host immune response (Arias & DuBois, 2017). Ren et al. developed a peptide-based ELISA (pELISA) using a specific peptide (amino acids 627–646) from the ORF2 protein of GAsV-1. This method exhibits high specificity and no cross-reactivity with other pathogens, providing an accurate, rapid, and economical detection tool for serological monitoring of GAsV-1 (Ren et al., 2023). Wang X, et al. expression of the C-terminal ORF2 protein of duck astrovirus for application in a serological test (Wang et al., 2011). Therefore, the capsid protein serves as a suitable antigen for establishing serological assays for astroviruses.

In this study, an indirect ELISA detection method was developed based on the N-terminal truncated capsid protein of GAsV. This region belongs to the highly conserved domain of the viral capsid protein (Bass & Qiu, 2000). The developed assay demonstrated excellent reproducibility and sensitivity, and showed no cross-reactivity with positive sera against other common goose viruses, indicating high specificity—a finding consistent with reports on other astroviruses. Moreover, the concordance rate between the indirect ELISA and Western blot reached 87.5%, suggesting that the capsid-based ELISA is applicable for testing clinical goose serum samples.

Subsequently, the newly developed indirect ELISA was used to test 288 clinical goose serum samples, revealing an overall GAsV antibody seropositivity rate of 38.19%. Samples collected from both Nanning and Hechi exhibited considerable positivity rates, indicating that GAsV is widespread among goose flocks in these regions. The capsid-based indirect ELISA established in this study represents a preliminary application, and its clinical utility warrants further evaluation. Given the absence of a commercially available reference ELISA kit, the present assay may have certain limitations. Therefore, future studies should involve the assessment of a larger number of clinical serum samples with well-documented background information.

In summary, this study established an indirect ELISA method for detecting GAsV antibodies using the GAsV capsid protein as the coating antigen. This method exhibits high specificity and sensitivity, making it suitable for clinical serological monitoring of GAsV and providing a powerful tool for understanding the epidemiological patterns of GAsV.

Declarations

Disclosure Statement

The authors report there are no competing interests to declare.

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Author Contribution

Conceptualization: Z.C., Y.Q.; Methodology: Z.C., Y.D.; Software: J.L.; Validation: S.W., N.L.; Formal analysis: M.H.; Investigation: Z.C.; Resources: K.O.; Data curation: Y.C.; Writing – original draft: W.Z.; Writing – review & editing: W.H.; Visualization: Y.Q.; Supervision: Y.Q.; Funding acquisition: Y.Q. All authors have read and agreed to the published version of the manuscript.

Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Figures

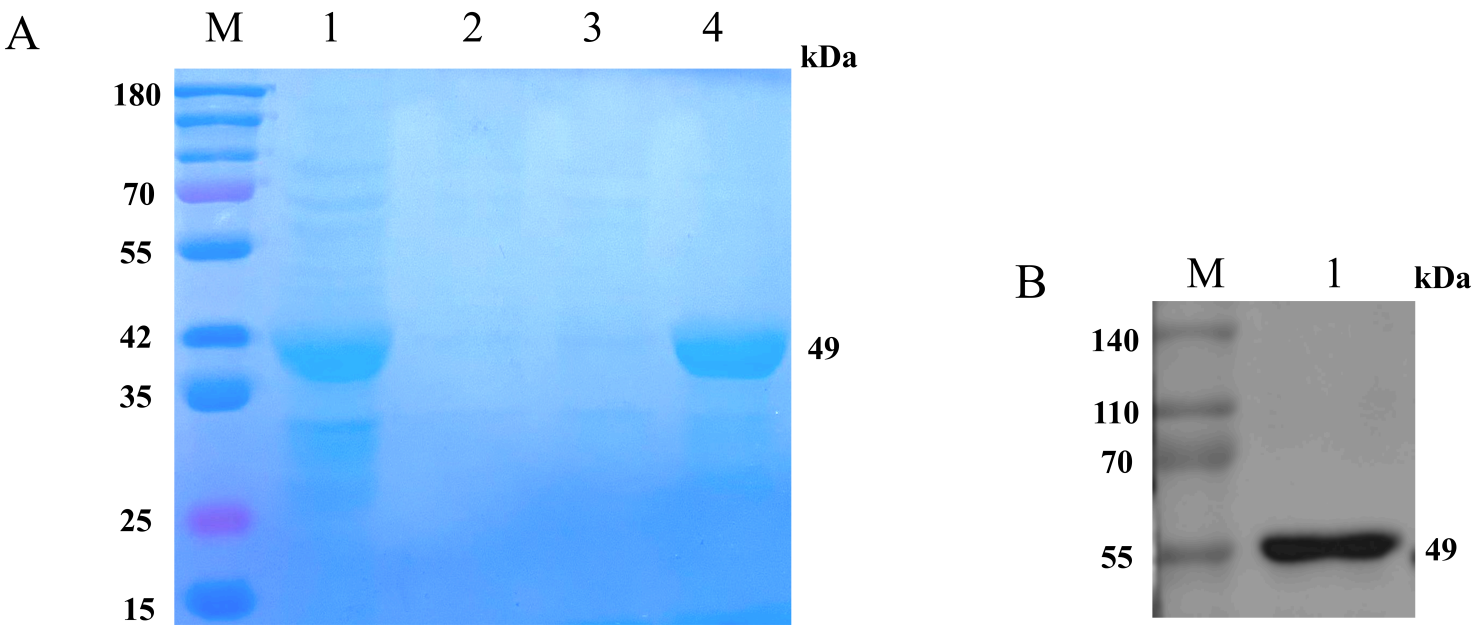


Figure 1

Figure 1

Expression and purification of GASTV-Cap-N protein. (A) The pET-32a-GASTV-Cap-N plasmid was transformed into *Escherichia coli* BL21. Expression of GASTV-Cap-N protein was detected by SDS-PAGE. M: protein marker; lane 1: total protein of whole bacterial cells after sonication; lanes 2 and 3: supernatant protein after sonication; lane 4: precipitated protein after sonication. (B) Using purified GASTV-Cap-N protein as the antigen, western blotting was performed with an anti-His tag mouse monoclonal antibody as the primary antibody to detect the GASTV-Cap-N protein.

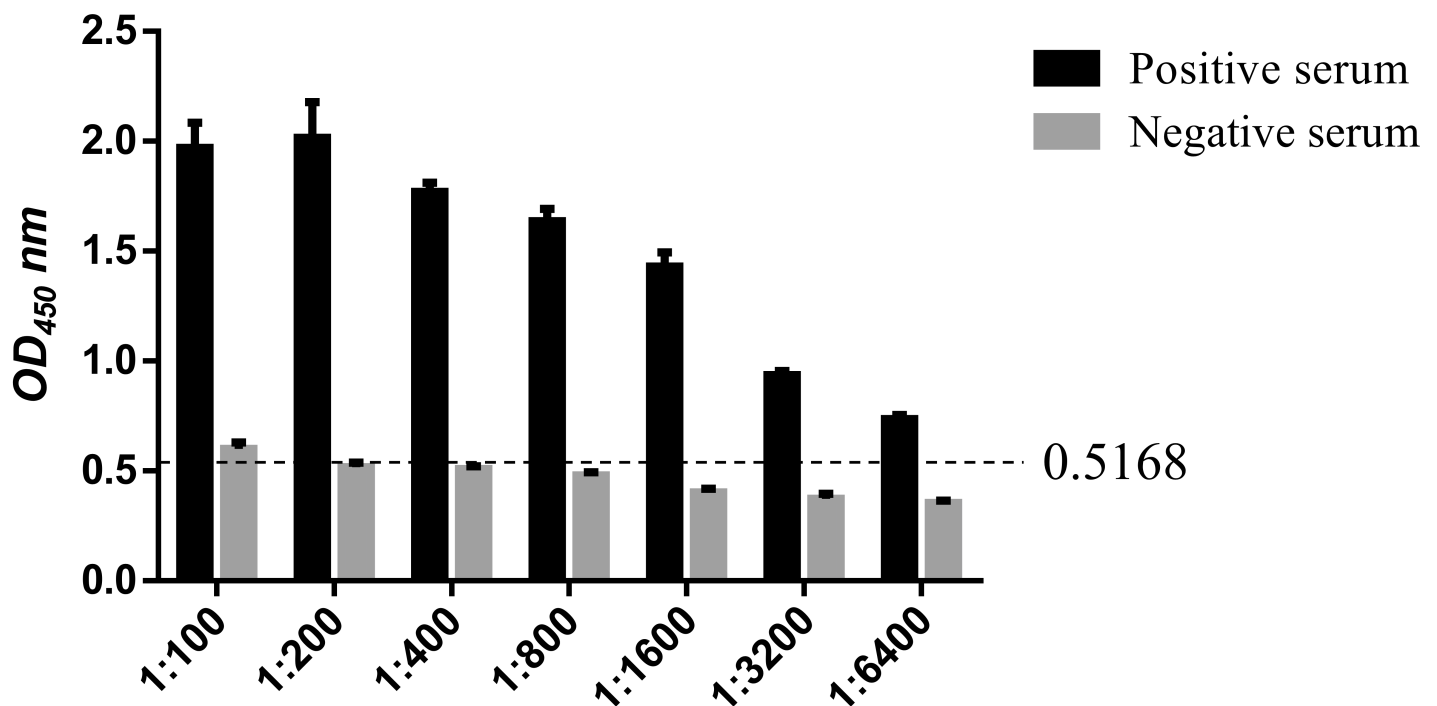


Fig. 2

Figure 2

Evaluation of the sensitivity of the GAsV-Cap-N-based indirect ELISA. Three GAsV-positive serum samples were serially diluted at ratios of 1:100, 1:200, 1:400, 1:800, 1:1,600, 1:3,200, and 1:6,400. Each serum sample at each dilution was tested in triplicate, and the OD₄₅₀ nm values were measured using the established indirect ELISA method.

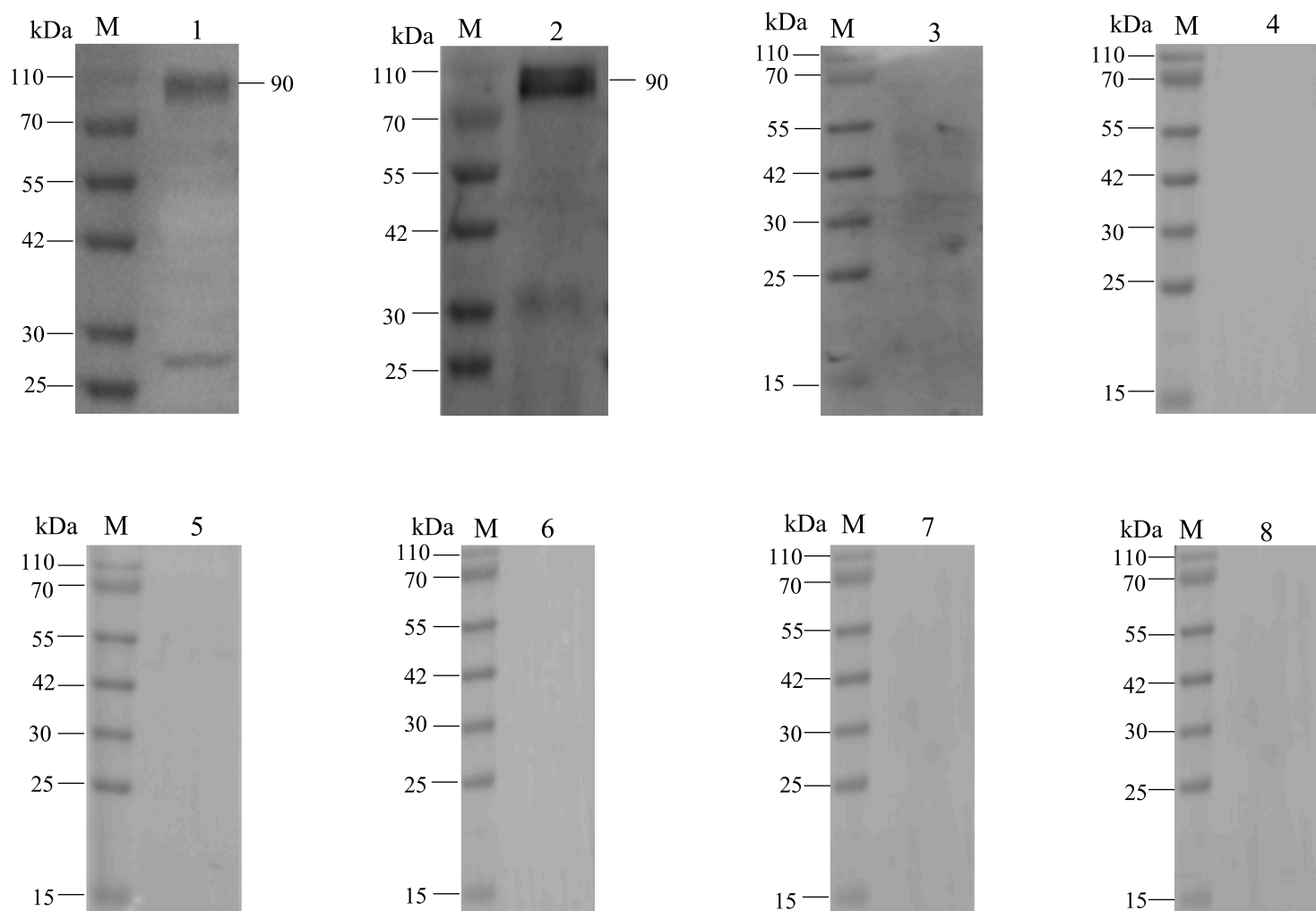


Fig. 3

Figure 3

Evaluation of the coincidence rate between the GAsV-Cap-N-based indirect ELISA and Western blot. Total proteins were collected from LMH cells infected with GAsV. Three goose serum samples that tested positive and five that tested negative by indirect ELISA were used as primary antibodies for Western blot analysis. Representative Western blots are presented. M, marker; lanes 1–3, positive serum samples; lanes 4–8, negative serum samples.