

Preparation and Preliminary Application of Egg Yolk Antibody against Duck-derived Astrovirus

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Abstract: In recent years, with the rapid development of China's waterfowl breeding industry, the number of common infectious diseases in ducks has been increasing, resulting in a high level of morbidity and mortality of duck diseases, which has seriously affected the development of waterfowl industry and caused huge economic losses. In this experiment, the preparation and preliminary application of egg yolk antibody against duck-derived astrovirus were studied. In this study, PCR identification and phylogenetic tree analysis were used to inoculate duck embryos after gradient dilution of the virus solution, and the death rate was counted. The median lethal dose (LD₅₀) of duck embryos was calculated according to the Reed-Muench method. To propagate duck-derived astrovirus, duck embryos were utilized. The virus was then rendered inactive using formaldehyde and combined with Freund's adjuvant for immunizing white feather laying hens. Hyperimmune eggs were subsequently gathered, and IgY was extracted through a method of water dilution-ammonium sulfate. The prokaryotic expression pCold-TF vector of DASTV spike protein was constructed, and the recombinant plasmid was transformed into *E. coli* for expression, which achieved efficient soluble expression of spike protein. SDS-PAGE electrophoresis was used to identify the amount and concentration of antibody and antigen protein, and indirect ELISA was used to detect the antibody titer of IgY produced by immunized hens. In the animal protection experiment, animal protective experiments and animal therapeutic experiments were carried out at the same time to verify the effect of egg yolk antibody. The results showed that the antigen could produce high titer egg yolk antibody. The titer of IgY detected by the indirect ELISA method established in this experiment was up to 1 : 1024. The virus titer was calculated to be 2×10^2 LD₅₀ / ml by Reed-Muench. In the protective experiment, ducklings were subcutaneously injected with 0.2ml egg yolk antibody. When challenged with 10LD₅₀ virus, the survival rate was 100 % after 15 days of challenge. When challenged with 20LD₅₀ virus, the survival rate was 75 % after 15 days of challenge. In the therapeutic experiment, when the ducklings were challenged with 10LD₅₀ virus and injected subcutaneously with 0.2ml IgY, the survival rate could reach 100 % after 15 days. When the ducklings were challenged with 20LD₅₀ virus and injected subcutaneously with 0.2ml IgY, the survival rate could reach 75 % after 15 days.

Keywords: duck-derived astrovirus, IgY, ELISA, Animal experiment

1. Introduction

In recent years, with the rapid development of China's waterfowl breeding industry, the number of common infectious diseases in ducks has been increasing, resulting in a high level of morbidity and mortality of duck diseases. Difficulties in clinical detection and control of these pathogens have affected the development of the waterfowl industry and caused great economic losses [1-5].

Therefore, the study of duck infectious diseases is particularly important. Duck astrovirus (DASTV) belongs to the genus Avian astrovirus in the family of Astroviridae. Among them, DASTV refers to the well-known duck hepatitis virus serotypes 2 and 3 [6]. In 2005, the ICTV 8th classification report renamed DHV-2 Duck astrovirus 1 (DASTV-I) and DHV-3 DASTV-2 [7-9].

Astroviruses were first discovered in ducks in 1965. In 1985, the Duck hepatitis virus 2 (DHV-2) was officially included in the family of astrovirus [10, 11], namely, Duck astrovirus 1. In 1969, researchers found that Duck hepatitis virus 3 renamed Duck astrovirus 2 in 2009 [12]. The symptoms

caused by DAsV-1 and DAsV-2 are similar to those caused by Duck hepatitis A virus (DHAV), both of which are shown as antennal arches, haemorrhagic and necrotic spots in the liver and spleen of the diseased ducks. Such disease is sudden, quick and widely spread. The mortality rate of ducklings aged two weeks infected with DAsV-1 can reach 50%. This may lead to the reduction of the production of eggs of adult ducks, thus seriously affecting the duck industry.

Immunoglobulins (Ig), also known as antibodies, are glycoproteins secreted by plasma cells stimulated by antigens from the external environment and play a major role in humoral immunity [13]. Immunoglobulins exist in body fluids and their main role is to combine with antigens, destroy pathogens and protect the body from being attacked by foreign pathogens [14]. Immunoglobulins are divided into IgA, IgM, IgG, IgE, and IgD. Eggs mainly contain IgY, IgM, and IgA, of which IgM and IgA exist in egg whites [15], and IgY only exists in the yolk, and is therefore called as yolk immunoglobulin or yolk antibody (IgY). It has been proved that immunoglobulins in the yolk only exist in the class of IgG [16]. Adopting the method of immunogenic immunisation of birds, yolk antibodies are extracted specifically from the yolk of produced eggs [17]. When laying birds are stimulated by external specific antigens, specific antigen antibodies produced by B lymphocytes will accumulate in the yolk and form egg yolk immunoglobulin (IgY) [18-20]. These preparation of yolk antibodies to become a hot topic for biotechnology studies in recent years.

The purpose of this study was to create a rapid and effective new biological product for the prevention and treatment of duck viral hepatitis (DVH), and to provide technical support for the comprehensive prevention and control of the disease.

2. Materials and Methods

The Dastv1 strains are all provided by Tianjin HLINTE Biotechnology Co., Ltd., and the clinical samples are collected from the suspected disease materials from the suspected disease duck farms in Heilongjiang.

Main Instruments: Ultra-clean bench (Model: BCN-1360B) is purchased from Beijing HDL APPARATUS Manufacturing Co., Ltd; Pressure steam sterilizer (Model: YXQ-LS-18S) is purchased from Shanghai BOXUN Medical and Biological Instrument Co., Ltd; Fully-automatic large-scale intelligent incubator is purchased from Weizhen Co., Ltd.

Main Reagents: TRIzol Reagent (Invitrogen); Random Primer (hexadeoxyribonucleotide mixture; pd(N)6) (Model: 3081) (Takara Biomedical Technology Co., Ltd.); 2xTaq PCR Starmix (Dye) (Model: A012- 01) (Takara Biomedical Technology Co., Ltd.); PrimeSTAR® HS DNA polymerase, pMD18-T vector, isopropanol (Tianjin Zonghengxing Industry and Trade Co., Ltd.); PrimeSTAR® HS DNA polymerase, pMD18-T vector and isopropanol (Tianjin Zonghengxing Industry and Trade Co., Ltd.); trichloromethane (Beijing Reagent); anhydrous ethanol (Tianjin Funing Fine Chemical Co., Ltd.); DEPC water (Biosharp Company);

3. Experimental Methods

3.1 Identification and Proliferation of Duck Astrovirus

The diseased ducks were dissected and 1cm³ of goose kidney tissue was taken. RNA is extracted by the Trizol method, CDNA is obtained through inverse transcription, and the virus detection is conducted through the RT-PCR method. The reaction system of PCR: 10xBuffer 2.5ul, dNTP 2ul, Dastv1F5'-gagcaagatcagaatgcgaga-3' 0.5ul, Dastv1R5'-catctgacagactctcc agtt-3' 0.5ul, Taq enzyme 0.25ul, ddH₂O 17.25ul, cDNA 2ul; The reaction procedure of PCR: pre-denatured at 95 °C for 5 min, 95°C 30 s, annealed at 95 °C for 30 s, extended at 72 °C for 30 s for 30 cycles, and extended at 72 °C for 10 min. The PCR products are added to the 1% agarose gel, and after electrophoresis, the sizes of the target fragments are checked by a UV analyzer to determine whether they are in line with the expectation. The gel is cut and sent to Comate Bioscience Co., Ltd. for sequencing, and the sequencing results is compared with the sequences in the NCBI database.

After the virus was identified, the internal organs of the diseased ducks were ground, centrifuged, and freeze-thawed to prepare a virus solution. The virus solution is diluted four times with sterilized 0.75% saline and then inoculated into 12-day-old duck embryos via the allantoic cavity. Each duck embryo is inoculated with 0.2 ml, incubated at 37°C, the embryos that died within 3 to 7 days are

collected and stored at 4°C. The internal organs of the duck embryos are separated, and the internal organs of the duck embryos and the allantoic fluid are stored at -20°C as the antigen of the inactivated vaccine.

3.2 Animal Regression Experiment

Ten 9-day-old ducklings without maternal antibody against DASTV are selected and randomly divided into two groups. Each group contain five ducklings, including the test group and the control group. Ducklings in test group 1 are inoculated intravenously with 0.2 mL of allantoic fluid collected in 2.1, while ducklings in the control group are injected with 0.2 mL of saline intramuscularly. The ducklings are isolated for 5 d. The dead ducklings are dissected to take the diseased materials, and the virus in the diseased material is detected by RT-PCR and sequenced by adopting the method in 2.1.

3.3 Genome Amplification and Sequence Analysis of DASTV Virus

The RNA in the diseased material is extracted and CDNA is obtained through the inverse transcription. The reaction system of PCR amplifies the whole genome of DASTV virus by section. The system is shown in 2.1, and the material is shown in Table 1. The PCR products is added to 1% agarose gel, and after electrophoresis, the size of the target fragment is observed by UV analyzer to determine whether the size is in line with the expectation. The gel is cut and sent to Comate Bioscience Co.,Ltd. for sequencing, and the sequence splicing is conducted by utilizing the Seq Man software of DNASTAR 7.1. The evolutionary tree is constructed through leveraging the Neighbor-joining (NJ) of MEGA.

Table1 Materials Used to Amplify the Strain

Name	Sequence	Point	Name	Sequence	Point
D1F	ccgaagtggcgagt	1-16	D6F	gaacacgccatcaatggtg	3713-3732
D1R	tcttcaactcccattgcatcc	562-581	D6R	catctgacagacttccagtt	4686-4707
D2F	caacggattgaagaagcaaatc	530-551	D7F	caactggagaagtctgtcaga	4684-4705
D2R	acaatccafftaacaagtaggtgg	1008-1030	D7R	ctggctcttcatcaagagtgga	5639-5660
D3F	gcacgtagcacattcaatgc	989-1008	D8F	tgatgaagagccaggaaaatgg	5647-5668
D3R	gtgacaaactctgaaccacg	1877-1896	D8R	tctgacgcaatccctactgt	6573-6592
D4F	gaattggattgacagcagg	1846-1866	D9F	ccaaatgtgaatgttcagc	6543-6562
D4R	attcatctgcagaccagcc	2897-2916	D9R	ctgtgaccacatggtgatt	7602-7621
D5F	agtcaaggaagtcacgcttg	2722-2741	D10F	ctctcgtccggttgagaaa	7429-7449
D5R	tcaggcccctcatctagtt	3740-3759	D10R	tttttttttttttttttttttttttttttaaatgcca	7715-7757

3.4 Measurement of Lethal Dose (LD50) in SPF Chickens

The allantoic fluid collected in 2.1 is diluted 10-fold with saline, and five dilutions of 1×10^{-3} , 1×10^{-4} , 1×10^{-5} and 1×10^{-6} are selected to inoculate 9-day-old duck embryos with 0.2 mL /pc. Each dilution is used to inoculate 5 embryos, and the embryos are observed for five consecutive days to record their deaths. The mortality rate of duck embryos inoculated with each dilution is calculated, and the LD50 of isolated strains of virus in the allantoic fluid is calculated by adopting the Reed-Muench method.

3.5 Preparation of Immunogen and Safety Experiment of Inactivated Vaccines

The visceral organs of duck embryos obtained are crushed with four times the volume of 0.75% saline by using a tissue crusher. The tissue suspension is filtered, the filtrate is mixed with the allantoic fluid, and the formaldehyde solution is added to make the final concentration reach 0.25%. The organs are put into shaking bed at 100r/min, and inactivated by 37% for 24 hours. Then they are left to stand for 24 hours, and preserved at 4°C. For long-term storage, 1% thiomersal should be added to each 100 ml of immunogenic solution. The inactivated virus liquid should be emulsified and mixed with Fuchsin complete adjuvant and stored at 4°C.

Ten 3-day-old ducklings are selected and randomly divided into the test group and control group. Each group contain 5 ducklings. In the test group, each duckling is injected subcutaneously with 2 mL of inactivated oil emulsion vaccine. In the control group, each duckling is injected subcutaneously with 2 mL of saline. The ducklings are observed for 14 consecutive days and their deaths are recorded.

3.6 Preparation of IgY

3.6.1 Animal Immunization

The 5-month-old healthy laying hens are selected to breed and observe for one week. When the situation is normal, the hens are inoculated with inactivated duck astrovirus vaccine. The injection part is the chest muscle, the basic immunity is 1.5 mL, the second immunity is 1.5 mL in the interval of 15 days, and the third immunity is 2.0 mL in the interval of 15 days. After the third immunity for 14 days, the sample eggs are selected to measure the specific antibody potency in the egg yolk liquid, and the potency is $\geq 1:128$. The high-immunity eggs are collected to be kept at 4°C for not more than 7 days.

3.6.2 Extraction of IgY and Measurement of Neutralization Potency

Eggs of each group after being immunized for 3 days are collected, labelled and stored in the refrigerator at 4°C. IgY is extracted and purified by acidified water dilution, saturated ammonium sulphate salting out and dialysis concentration. The extracted IgY is first diluted 2-fold, and 1 mL of IgY at five dilutions of 2^{-2} , 2^{-4} , 2^{-8} , 2^{-16} and 2^{-32} is mixed with equal amounts of virus-containing allantoic fluid collected in 2.2, and neutralized at 37 °C for 1 hour. 9-day-old duck embryos are inoculated with 0.2 mL of the dilution, and each dilution is inoculated into five duck embryos. Another five duck embryos are inoculated with 0.2 mL/egg of saline, and taken as a control. The duck embryos are observed for 5 consecutive days and their deaths are recorded. The neutralization potency of IgY is measured by the Reed-Muench method.

3.7 Test of IgY

(1) Test of Physical Stability: take 30 ml of IgY and put it in a 50 ml centrifuge tube, let it stand at the room temperature for 24 hours, and observe its colour and transparency, and whether there is precipitation. (2) Sterility Test: In the ultra-clean table, suck 0.5 ml of IgY with a sterilized gun tip and inoculate it into LB culture medium, smear it evenly with inoculation ring and incubate it at 37°C for 24 hours, and observe whether there is any growth of microorganisms. (3) Safety Test: Use a syringe to inject 0.2 ml of IgY subcutaneously into the immunized ducklings and observe whether there is any abnormality in the ducklings. (4) Detect the purity of IgY by adopting the SDS-PAGE method, The gel configuration system is as follows, separator gel: deionized water 2.3 mL, 30% acrylamide 5ml, PH 8. 8 Tris-HCl(1.5M)2.5ml, 10%SDS 0.1ml, 10%APS 0.1ml, TEMED 0.004ml; espresso: deionized water 5mL, 30% acrylamide 3.4ml, PH 6. 8 Tris-HCl (1.5M) 2.5ml, 10%SDS 0.1ml, 10%APS 0.05ml, TEMED 0.005ml.

3.8 Preparation of Fibrillar Proteins

PCR amplification is conducted on the fibronectin gene. The reaction parameters are: cDNA 1 μ L, upstream 5'-GGATCCgttggtataagataacagtgttaggc-3' (10 μ mol/L) and downstream primers 5'-GTCGACggcagcttggtgttctg-3' (10 μ mol/L) 0.5 μ L, 2 \times PCR mix 10 μ L, ddH₂O 8 μ L; The reaction procedure of PCR is: 94 °C for 2 min; 94 °C for 30 s, 56 °C for 45 s, and 72 °C for 45 s for 30 cycles; and 72 °C for 10 min. After the PCR products are recycled, the products are double digested with pCold+tf vector. After the digested products are recycled, they are connected with T4 ligase to construct the reorganized plasmid pCold-TF, and be transformed into E. coli DH5 α receptor cells. Then they are stored at low temperature for spare parts after being identified correctly by the bacterial PCR, double digest and DNA sequencing. Take a little bacteria into LB medium (Amp⁺, 50 mg/mL), and incubate it at 37 °C overnight. Take 2 mL of fresh bacterial solution and add it into a conical flask containing 200 mL of LB medium, incubate it at 37 °C, 225 r/min until the OD value reaches 0.6 to 0.8, then add IPTG at a final concentration of 1.0 mmol/L to induce expression for 2 hours. At 5 000 r/min, centrifuge it for 10 min, and discard the supernatant. Purify the remaining bacteriophage and detect the protein by SDS-PAGE.

3.9 Measurement of Potency of Anti-duck Astrovirus IgY

Eggs of each group after being immunized for 3 days are collected, and yolk antibody is extracted separately, the antibody titer was determined by the indirect ELISA method established in our laboratory. Specific as follows:the antigen protein coating amount should be 1 ng/ μ L overnight coating at 4°C, the dilution ratio of yolk antibody should be 1:200. The sealing solution used should be PBST solution with 3% BSA. It should be sealed at 37°C for 2 hours. The primary antibody should be

incubated at 37°C for 45min, and the secondary antibody should be incubated at 37°C for 45min.

3.10 Animal Protective and Therapeutic Experiments

Twenty-four newly fledged ducklings are selected, grouped, injected with IgY and challenged as shown in Table 2, and the clinical characteristics are observed and recorded.

Table 2 Experimental Grouping of Gosling Protection

groups	3 days old	5 days old	6 days old
1 (of 10)	hypodermic injection 0.2ml IgY	hypodermic injection 0.2ml IgY	hypodermic injection 20LD50
2 (of 10)	hypodermic injection 0.2ml IgY	hypodermic injection 0.2ml IgYL	hypodermic injection 10LD50
3 (of 4)	hypodermic injection 0.2ml saline (medicine)	hypodermic injection 0.2ml saline (medicine)	hypodermic injection 10LD50

Twenty-four newly fledged ducklings Are selected, grouped, injected with IgY and challenged as shown in Table 3, and the clinical characteristics are observed and recorded.

Table 3 Therapeutic Experimental Grouping of Goslings

groups	6 days old	9 days old
1 (of 10)	hypodermic injection 20LD50	hypodermic injection 0.2ml IgY
1 (of 10)	hypodermic injection 10LD50	hypodermic injection 0.2ml IgY
1 (of 4)	hypodermic injection 10LD50	hypodermic injection 0.2ml saline (medicine)

4. Results

4.1 Identification of Duck-derived Astrovirus

After the inverse transcription of the extracted RNA, goose-derived astrovirus is amplified with specific primers. Based on the result of 1% agarose gel electrophoresis, it can be clearly seen that there is a bright band between 250bp and 500bp, which is in line with the expected 319bp. It is sent to Comate Bioscience Co., Ltd. for sequencing and compared with the data in the NCBI database, and it is identified as duck-derived astrovirus.

4.2 Construction of Evolutionary Tree and Virus Homology Analysis

The results of gene homology analysis of the isolates showed that the nucleotide identity between the isolates and the virulent strains D51, D390, DA06, DA07, DA08, DA93, D120, and WF1201 is from 90.2% to 95.2%. Among them, the similarity with the virulent HB2015, SDWF, and SDZZ is higher at 97.4% to 98.5%, with smaller differences. This is shown in Figure 1.

The phylogenetic tree is constructed by using MEGA with NJ method, and the analysis of the evolutionary tree showed that the isolated duck astrovirus gathers together with OL652659.1, KY646154.1, FJ919227.1, FJ919227.1, FJ919225.1, FJ919225.1, MW217578.1 and OL652659.1 in GenB ank. This indicates that the isolate is DASTV-I and is named ZYL.

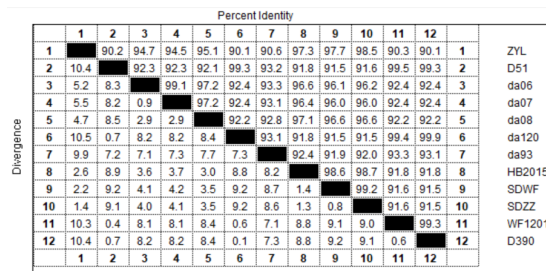


Figure 1 Results of Sequence Homology Analysis

4.3 Animal Regression Experiment

The results showed that all ducklings in the test group died within 72 hours, while all ducklings in the control group survived. The dead ducklings in the test group had their heads tilted back and were in the posture of ankylosis, and the liver was enlarged with haemorrhagic spots as shown in the figure. Viral RNA is extracted from the liver tissue of the dead ducklings, and the target bands are amplified by RT-PCR. The sequencing results are in line with those in 4.1, indicating that the isolate is highly pathogenic to 9-day-old ducklings.

4.4 Status and Egg Production of Immunized Chickens

After inactivation with formaldehyde, the virus solution was emulsified with the adjuvant to become milky white. Laying hens are in good mental state after being immunized. The egg production decreases after the chickens are immunized. This is a normal phenomenon of stress reaction or stimulation of immune complex in chickens in the process of being immunized, and the egg production can be resumed in 2 to 3 days, and the average daily egg production reaches 0.9.

4.5 Virus Toxicity

According to the data in Table 4, the LD₅₀ of the isolate in the allantoic fluid on 10-day-old duck embryos is calculated by the Reed-Muench method to be 2×10^2 LD₅₀/ml.

Table 4 Results of LD₅₀ Measurement of Isolates

dilution	Number of survivors/each	Deaths/each	Cumulative number of survivors/each	Cumulative number of deaths per piece	death rate	Mortality rate/%
1×2^{-3}	0	5	0	14	14/14	100
1×2^{-4}	1	4	1	9	9/10	90
1×2^{-5}	2	3	3	5	5/8	62.5
1×2^{-6}	3	2	6	2	2/6	25

4.6 IgY Neutralization Potency

According to the data in Table 5, the neutralization potency of IgY is calculated by the Reed-Muench method to be 1:256.

Table 5 Measurement Results of the Neutralization Potency of IgY

dilution	Survival number / piece	Number of deaths / pieces	Cumulative number of survivors/each	Cumulative number of deaths / piece	survival ratio	protection ratio
1×2^{-2}	5	0	20	0	20/20	100
1×2^{-3}	5	0	15	0	15/15	100
1×2^{-4}	4	1	10	1	10/11	90.9
1×2^{-5}	4	1	6	2	6/8	75
1×2^{-6}	2	3	2	5	2/7	28.6

4.7 Fibrillar Protein SDS-PAGE

After SDS-PAGE electrophoresis and being dyed with Coomassie Brilliant Blue stain and destained with decolourising solution, and the gel electrophoresis results are reflected by the size of the proteins around 60kDa.

4.8 Test of Yolk Antibody

(1) Results of the test of physical stability: the purified yolk antibody becomes the light yellow transparent liquid, and after standing, a small amount of white precipitate can be generated, and it can be dissolved after slight shaking. (2) Sterility test: the antibody is inoculated in LB medium and incubated at 37°C for 24 hours. There is no growth of microorganisms such as bacteria and moulds. (3) Safety test: After immunizing the ducklings with the antibody under the condition of subcutaneous

injection in the neck, the ducklings are in good mental state and no adverse phenomenon occurs.(4) After SDS-PAGE electrophoresis, and being dyed with Coomassie Brilliant Blue stain and destained with decolourising solution. There are two distinct bands in the figure, and the size of the larger one for protein is about 50 kDa, and the size of the other is about 36 kDa.

4.9 Measurement of Anti-duck-derived Astrovirus IgY Potency

After collecting the immunized eggs and separating the egg whites and yolks, the antibody potency in the yolks is measured by indirect ELISA method. The antibody potency in the yolks is $\geq 1:256$ after the eggs being first immunized for 3 days, and the antibody in the yolks reaches the highest level after the third immunization for 3 days, and the antibody potency in the yolks is $\geq 1:1024$. It can be seen that the inactivated vaccine of the invention can stimulate the laying hens to produce a high level of IgY.

4.10 Protective Experiment on ducks and Therapeutic Experiment on ducks

Protective Experiment: the ducks are subcutaneously injected with 0.2 ml of IgY. After being immunized twice, when the ducks are subcutaneously injected with a viral load of 10 LD₅₀, the survival rate of the ducks can reach 100% after 15 days. When the ducks are subcutaneously injected with a viral load of 20 LD₅₀, the survival rate of the ducks can reach 80% after 15 days. The ducks of the control group died three days after being injected. Therapeutic Experiment: when the ducks are subcutaneously injected with a viral load of 10 LD₅₀, the survival rate of the ducks can reach 90% if the ducks are subcutaneously injected with 0.2ml IgY after 3 days. When the ducks are subcutaneously injected with a viral load of 20 LD₅₀, the survival rate of the ducks can reach 70% if the ducks are subcutaneously injected with 0.2ml IgY after 3 days. The ducks of the control group died three days after being injected.

5. Discussion

In order to better prevent and treat DHV, we developed an egg yolk antibody against DASTV. In the preparation of DASTV virus immunogen, this study adopts the formaldehyde inactivation method, adds 0.1% formaldehyde solution to the virus solution, puts the solution in the incubator at 37 °C, inactivates it for 16 hours, vibrates and mixes it each 4 hours, and conducts an inactivation test on it until it is completely inactivated. The prepared immunogen is emulsified with white oil adjuvant and becomes water-in-oil. After being detected, the physicochemical properties of the immunogen are stable and there is no growth of microorganisms such as bacteria and moulds.

In this experiment, the water dilution-ammonium sulfate precipitation method is adopted to extract and purify the yolk antibody, and the results of SDS-PAGE showed that yolk antibody with high purity can be obtained. The potency of the prepared yolk antibody is measured by the indirect ELISA method, and can reach 1: 1024. The indirect ELISA method created in the experiment can be used not only to detect the yolk antibody, but also to evaluate the protective effect of the vaccine, and enhance the immunization that fails to produce the expected result. Meanwhile, this method can also be used to find the early infection of duck-derived astrovirus, and to isolate and treat the diseased ducks in time, thus reducing the economic losses of duck factories.

In the animal protective and therapeutic experiments, ducklings are selected for the experiments. Since the ducklings are fragile, attention should be paid to heat preservation and rearing in separate cages in the experiments, so as to avoid the influence of external factors. In the protective and therapeutic experiments on ducklings, it is proved that the purified yolk antibody has good protective and therapeutic effects.

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