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Article

Immunogenicity of Minhai 13-valent pneumococcal polysaccharide conjugate vaccine in experimental mice

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SUMMARY

Diseases caused by pneumococci are a global public health problem. The widespread use of antibiotics has led to a sharp increase in the drug resistance of pneumococci, making research on pneumococcal vaccines highly relevant. Pfizer's 13-valent pneumococcal conjugate vaccine (PCV13) is effective; however, its use in low- and middle-income countries is challenged by cost and logistical difficulties in vaccine transportation, storage, and management. Therefore, there is an urgent need to develop efficient, convenient, and affordable vaccines in these countries. An alternative pneumococcal vaccine is produced by Minhai, but its effectiveness is not well understood compared to Pfizer's vaccine. Differences in bacterial strains, vaccine carrier proteins, and conjugation processes may lead to different immune protections against pathogenic pneumococci. We hypothesized that the pneumococcal conjugate vaccine produced by the Minhai company would elicit significant immunogenicity in experimental mice, though potentially lower than that of the Pfizer vaccine. To test this hypothesis, we gave the Pfizer or Minhai vaccine to mice. Then we used an enzyme-linked immunosorbent assay (ELISA) to detect the antibody geometric mean titer (GMT) of serum IgG antibodies in the vaccinated mice. Although there were slight differences in the antibody titers against the 13 serotypes, these differences were not statistically significant, and the antibody titers for all serotypes met the requirements for immune protection. We concluded that the 13-valent pneumococcal conjugate vaccine produced by the Minhai company elicits a robust immune response in mice.

INTRODUCTION

Pneumococci are Gram-positive bacteria whose capsule is their main virulence factor. The capsular polysaccharides exhibit antigen specificity and can be recognized by specific antibodies or immune cells, leading to a targeted immune response. Based on the antigenic structure of the capsular polysaccharide, pneumococci are divided into 91 serotypes (1, 2). Research shows that over 80% of invasive diseases caused by pneumococci worldwide are due to 30 serotypes out of all the serotypes of the pneumococcus (3). Thirteen serotype strains are responsible for 70% to 75% of invasive diseases globally (4). Pneumococcal bacteria mainly exist in the nasopharynx of healthy individuals and are a major causative agent of bacterial lobar pneumonia, meningitis, community-acquired pneumonia, and bronchitis (5). Community-acquired pneumonia is a common complication of influenza (6). In 2005, the World Health Organization (WHO) reported that 1.6 million people die from pneumococcal infections annually (7). Diseases and complications caused by pneumococcal infection seriously affect human health and are a leading cause of death in infants, children, and the elderly (8). Human immunodeficiency virus (HIV) infection and immune deficiency-related diseases significantly increase the incidence of pneumococcal pneumonia.

The overuse of clinical antibiotics has led to drug resistance in over 96% of pneumococcal strains, making the treatment of pneumococcal pneumonia challenging (9). In the latest WHO antibiotic research and development plan, Streptococcus pneumoniae is on the priority pathogen list for new antibiotic research and development, highlighting the severity of the treatment situation (10). The use of pneumococcal vaccines has become the most effective method for preventing pneumococcal diseases (11). The history of pneumococcal vaccines dates back nearly 100 years, having gone through multiple stages, including monovalent and multivalent whole-cell vaccines and capsular polysaccharide pneumococcal conjugate vaccines of various serotypes (12). In recent years, the widespread use of the 13-valent pneumococcal conjugate vaccine, produced by Pfizer in the United States, has significantly reduced the incidence of pneumococcal-related diseases, with a 39% decrease in infections caused by vaccine-covered serotypes (13, 14). The Pfizer vaccine entered China in 2016. However, due to cost, storage, and transportation issues, the vaccination rate of the target population in China is only about 7% (15). Approximately 10 million newborns and over 200 million people over 60 years old in China need to be vaccinated against pneumococci annually (16). The vaccination coverage rate of pneumococcal conjugate vaccine (PCV) among children in China is far lower than the global average (17). In 2010, Pfizer introduced the world's first 13-valent pneumococcal conjugate vaccine (PCV13). Previous studies have compared Pfizer PCV13 with the 23-valent polysaccharide vaccine (PPSV23) (18-20). The results from these studies showed no statistically significant difference in the GMT of serotype antibodies of 3, 5, 14, and 19F compared to PPSV23 (18,20). For other common serotype antibodies, PCV13 was higher than PPSV23. Wang Ting-Ting et al. compared Minhai PCV13 with Pfizer PCV7. Their results showed that Minhai PCV-13 had the same immunogenicity as Pfizer PCV-7 against the 7 common pneumococcal serotypes ($t = 0.004$, $p > 0.05$) (21).

Our study looked at the effectiveness of the Minhai vaccine compared to Pfizer's PCV13. We hypothesized

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that the pneumococcal conjugate vaccine produced by the Minhai company would elicit significant immunogenicity in experimental mice, though potentially lower than that of the Pfizer vaccine. To test our hypothesis, we gave the Minhai or Pfizer vaccine to mice and then looked at the serum IgG antibody levels in each group using an enzyme-linked immunosorbent assay (ELISA). Our results showed no significant differences in immunogenicity and safety between the two vaccines. This suggests that the Minhai pneumococcal conjugate vaccine has demonstrated comparable immunogenicity and safety to the well-known Pfizer PCV13 vaccine in experimental mice. The results, measured by serum IgG antibody levels, show no significant differences in effectiveness between the two vaccines. This indicates that the Minhai vaccine has the potential to be a viable alternative to the Pfizer vaccine. This is particularly important in some developing countries, where, due to production and distribution costs, the Minhai vaccine may offer a more cost-effective option for countries or health systems looking to expand vaccination coverage against pneumococcal diseases.

RESULTS

Safety of the Minhai vaccine

The safety of the Minhai vaccine was assessed by monitoring the diet, weight, and activity of the mice. Before vaccination, the average weight of the mice was 13.83 ± 0.73 g, with no statistically significant difference between these groups ($p = 0.929$). After vaccine administration, both groups were closely monitored. All mice survived, displayed normal sensitivity, had shiny fur, and ate regularly. The average weight of the mice increased to 17.85 ± 0.65 g, representing an average weight gain of 29.1% across two groups (**Figure 1**). No statistically significant difference in body weight was observed between the two groups ($p = 0.956$). These results suggest that both vaccines demonstrated good safety profiles.

Vaccination success rate

To ensure the accuracy of the experiment and the success of vaccination for each vaccinated mouse, we measured the concentration of carrier proteins in the serum of every vaccinated mouse. The Minhai group was tested for the concentration of diphtheria toxin (DT) carrier protein, represented by an optical density (OD) value, while the Pfizer group was tested for the concentration of the diphtheria toxin mutant (CRM197) carrier protein. Our results showed that all mice in both groups exhibited detectable levels of the respective carrier proteins, with OD values significantly above the baseline threshold (**Table 1**). These findings show that the carrier proteins CRM197 and DT were detected in all mice. Since the vaccines administered to both groups of mice contained carrier proteins, we considered the vaccination success rate to be 100%.

Antibody levels and data analysis

To compare the immunogenicity of the two vaccines, the serum antibody titers of various types were measured via ELISA. Geometric mean titers (GMTs) for the Minhai and Pfizer groups, along with OD values for the negative control group, were collected (**Tables 2 and 3**). The normality test of the GMT values for all samples showed that the serum type 5 antibodies followed a non-normal distribution, while the others followed a normal distribution (**Table 4**). An independent-sample t-test was conducted on the GMTs of the two groups. Among the 13 serotypes of antibodies, no significant differences ($p > 0.05$) were observed in the GMTs between the Minhai and Pfizer groups for all serotypes except serotype 5 ($p = 0.007$) (**Table 5**). The GMT of serotype 5 in the experimental group was 9000.25 titer units, which was significantly higher than that of the control group, 5341.09 titer units ($p < 0.05$, non-parametric test). Given that we were assessing the immunogenicity of the experimental group, the

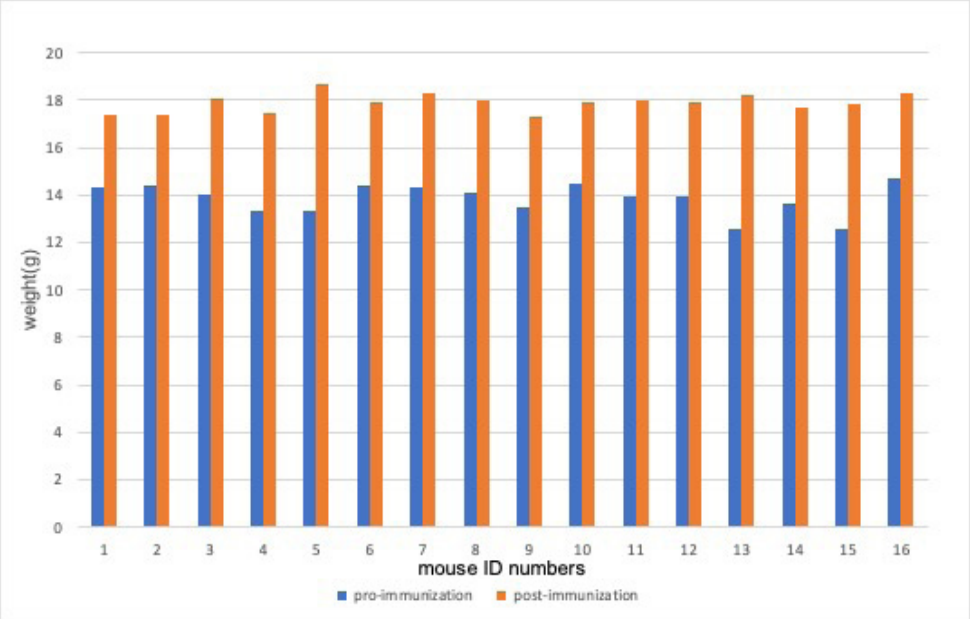

Figure 1. Body weights of mice pre- and post-immunization. Mean ± SD of body weight (n=8 per group). Mice were weighed at AGE prior to receiving one of the vaccines and then at AGE, 35 days, after receiving the vaccine. The average increase in body weight after immunization was 29.1%. No statistical difference was observed between the Minhai and Pfizer groups (t-test $p>0.05$). Mouse numbers 1-8 represent the Minhai group; mouse numbers 9-16 represent the Pfizer group.

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Mouse number	1:2000	1:4000	1:8000	1:12000	1:16000	1:32000	Negative	Blank
1	4.573	4.327	3.756	3.043	2.533	1.735	0.205	0.148
2	4.455	4.052	3.274	2.665	2.359	1.632	0.173	0.152
3	3.757	2.872	1.969	1.479	1.253	0.797	0.197	0.153
4	4.185	3.314	2.396	1.919	1.598	0.957	0.182	0.137
5	4.299	3.577	2.791	2.248	1.980	1.349	0.162	0.150
6	4.103	3.595	2.744	1.926	1.989	1.295	0.158	0.130
7	4.204	3.493	2.866	2.292	2.060	1.345	0.157	0.128
8	3.260	2.211	1.508	1.189	0.960	0.584	0.176	0.119
9	3.865	2.888	2.036	1.438	1.267	0.791	0.147	0.147
10	3.417	2.471	1.687	1.203	1.030	0.657	0.146	0.091
11	2.850	1.927	1.348	0.980	0.834	0.511	0.135	0.093
12	3.819	2.985	2.107	1.627	1.351	0.833	0.157	0.095
13	4.144	3.295	2.314	1.785	1.506	0.979	0.145	0.095
14	4.278	3.351	2.421	1.763	1.533	0.978	0.137	0.092
15	3.424	2.640	1.813	1.260	1.111	0.703	0.132	0.098
16	3.589	2.970	2.039	1.452	1.241	0.770	0.151	0.092

Table 1. CRM197 and DT titers for Pfizer and Minhai groups in mice. OD values of CRM197 and DT titers in the serum of mice in the control group. Six different dilutions were tested to determine whether the vaccination is successful, with success being defined as whether the administration process is successful. Mouse numbers 1-8: CRM197 in the Pfizer group; mouse numbers 9-16: DT in the Minhai group.

Sample Code	1	3	4	5	6A	6B	7F	9V	14	18C	19A	19F	23F
1	9.16	4.30	12.0	4.39	12.5	5.59	5.11	10.8	4.09	10.7	11.3	5.49	3.34
2	7.36	3.08	11.6	3.68	11.0	4.34	4.01	9.19	3.72	9.94	9.66	3.17	1.96
3	4.33	3.93	11.4	3.34	9.83	2.77	2.62	10.1	3.18	6.75	7.57	4.83	4.82
4	9.39	4.06	12.9	6.18	10.7	5.79	2.58	11.1	1.48	11.5	9.70	11.7	1.96
5	7.87	2.34	14.3	4.17	15.4	3.00	5.62	10.6	16.7	13.9	12.0	7.09	3.68
6	1.64	5.57	13.5	5.91	12.0	5.72	2.61	11.3	5.61	11.1	8.65	6.93	5.33
7	6.47	7.74	15.5	10.1	13.1	6.49	3.75	14.5	10.4	12.4	12.9	9.95	6.17
8	6.09	1.94	10.1	5.01	4.75	2.20	5.57	8.29	1.21	8.27	7.71	7.71	3.19
9	6.49	4.75	13.7	13.7	22.8	5.69	4.07	14.8	1.69	8.60	15.6	6.46	0.76
10	16.5	3.19	11.7	8.35	4.33	3.84	5.38	5.30	4.71	11.0	12.6	9.37	4.18
11	2.40	3.80	8.97	8.32	1.64	3.53	5.58	8.87	0.43	8.01	10.1	1.87	3.03
12	5.53	3.35	8.89	9.09	5.39	5.97	1.86	9.09	1.00	8.66	9.99	5.12	4.51
13	9.21	4.26	10.5	9.34	1.08	1.04	1.57	8.22	3.99	10.5	8.50	7.81	0.84
14	5.35	7.14	10.6	8.88	1.68	1.45	1.99	10.4	3.39	12.2	10.9	7.01	1.20
15	13.4	0.85	11.8	7.62	10.9	0.73	3.61	11.1	0.80	8.31	9.32	5.64	5.16
16	10.1	0.97	11.3	6.70	19.5	0.89	1.72	9.66	1.60	9.60	9.26	10.4	0.82

Table 2. GMT (x10⁴) of the Minhai and Pfizer group. The geometric mean titers (GMT) of antibodies for different serotypes in the Minhail and Pfizer groups. The GMT values are presented for each serotype across the two groups. Mouse numbers 1-8 represent the Pfizer group; mouse numbers 9-16 represent the Minhai group.

difference in serotype 5 GMTs can be considered negligible. Overall, our results indicate that the experimental group exhibited robust immunogenicity.

Correlation between carrier proteins and antibodies

The carrier protein of Pfizer's 13-valent pneumococcal vaccine is a diphtheria toxin (DT) variant (CRM197), while Minhai's 13-valent pneumococcal vaccine uses two carrier proteins: DT for serotypes 3,4, 6B, 7F, 14, and 18C, and tetanus toxoid (TT) for serotypes 1, 5, 6A, 9V, 19A, 19F, and 23F. To study the correlation between antibodies and carrier proteins, we compared the antibodies corresponding to DT and TT in the Minhai group to the serotype antibodies corresponding to CRM197 in the Pfizer group. Our results showed no significant difference in the serum antibodies corresponding to DT and TT and the CRM197 carrier protein ($p > 0.05$, **Tables 6 and 7**). However, a significant difference in the experimental group was observed in the serotype antibodies corresponding to DT and TT ($p = 0.003$). The antibody GMT corresponding to TT was significantly higher than that of DT.

DISCUSSION

In our study, the GMT of antibodies was compared between mice who had received a Minha or a Pfizer pneumococcal vaccine (p=0.119 - 0.860). The antibody levels of the two groups were comparable, except for the serum type 5 antibodies, which showed a significant difference between the two groups ($p =0.007$). The antibody levels of serotype 5 in Minhai's PCV13 were higher than those of Pfizer's. Since the type, structure, and purity of carrier proteins can affect the immunogenicity and safety of conjugate vaccines, and the production process of vaccines directly influences the binding efficiency and stability between antigens and carrier proteins, the differences observed may be related to variations in the production processes. In our experiments, we indeed observed differences in carrier proteins, which led to variations in antibodies. Therefore, in future research on human vaccines, the selection of carrier proteins will be an important direction. With the rapid development of artificial intelligence, significant progress is bound to be made in the screening of carrier proteins.

The main factor affecting this experiment is the small sample size, which results in the data of each experimental animal accounting for a large proportion of the total experiment, reaching 12.5%. In the experimental group, the DT value and antibody level are positively correlated. The low DT value of the third mouse in the experimental group resulted in low overall data for this mouse, which had a certain impact on the experimental results. The possible reason is related to individual absorption differences in mice. The solution is to detect the carrier protein content of the experimental animals and increase the number of samples in the experiment to reduce the proportion of individuals in the experiment.

In this study, we used an ELISA to detect antibody levels following pneumococcal vaccine immunization. Although ELISA is a quantitative detection method known for its high sensitivity and specificity, and it offers several advantages such as ease of standardization, convenient operation, and rapid results, the antibodies detected by ELISA do not necessarily reflect their opsonic-killing ability (22,23). In contrast, the opsonophagocytic assay (OPA) is currently the most effective method for evaluating the bactericidal capacity of pneumococcal vaccines. However, due to its high cost and complexity, OPA is primarily used by vaccine manufacturers

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Group	1	3	4	5	6A	6B	7F	9V	14	18C	19A	19F	23F
1	2.40	2.78	1.86	1.31	2.50	2.76	1.78	1.66	2.05	1.19	2.14	2.58	3.05
2	2.35	2.67	1.91	1.46	2.54	2.76	1.82	1.64	3.92	1.17	2.02	2.97	2.81
1	2.56	3.43	1.91	1.71	3.12	2.79	2.12	2.07	2.51	1.50	2.38	2.61	2.57
2	2.67	3.33	2.10	1.80	3.19	2.85	2.17	2.22	2.42	1.48	2.69	2.52	2.67
1	1.97	2.47	1.48	1.19	2.18	2.33	1.59	1.60	1.87	1;20	1.96	1.99	2.85
2	2.20	2.40	1.50	1.36	3.05	2.27	1.63	1.66	1.90	1.09	2.05	2.29	2.19
1	1.32	1.95	1.07	0.82	1.50	1.65	1.17	0.84	1.23	0.76	1.34	1.42	2.10
2	1.41	1.98	1.07	1.02	1.70	1.61	1.16	0.90	1.21	0.74	1.36	1.53	2.03

Table 3. OD ($x10^{-1}$) of negative group (dilution 1:1000). The optical density (OD) values of the negative control group at a dilution of 1:1000 for each serotype. These values are used to calculate the cutoff value to determine antibody positivity. Numbers 1 represent the Minhai group; numbers 2 represent the Pfizer group.

and testing institutions to assess the immunogenicity of pneumococcal vaccines (24).

Nevertheless, ELISA was chosen as the primary detection method in this study based on its practical convenience and extensive application experience. Numerous experiments conducted in China have demonstrated a high degree of consistency between antibody titers determined by OPA and ELISA, with results from both methods showing good agreement (18). Therefore, we believe that the use of ELISA to detect antibody levels in this study is reasonable and unlikely to affect the interpretation of the results due to the absence of OPA testing.

Currently, there are only a handful of companies in the world producing 13-valent pneumococcal conjugate vaccines, including Pfizer in the United States and several companies in China. Pfizer was the first company globally to develop this vaccine, while the Chinese vaccine has incorporated certain modifications based on the original production process. Our study demonstrated that there is no significant difference between the two vaccines in their core function, namely immunogenicity. However, the domestic vaccine demonstrates advantages in production efficiency, cost-effectiveness, transportation, storage, and suitability for use within China. Notably, domestically produced vaccines are distributed directly to epidemic prevention stations without intermediate steps, which represents a distinct logistical advantage. The data obtained from this study provides scientific evidence to support the use and selection of vaccines for the Chinese population. It is important to note that this study was conducted on animals, and while Minhai's PCV13 has shown promising safety and immunogenicity, further clinical data will be necessary to validate its efficacy in humans.

Looking ahead, one promising area of research involves the use of alternative carrier proteins, such as ferritin, which has shown remarkable potential in enhancing vaccine immunogenicity. Studies have demonstrated that ferritin-based vaccines can induce neutralizing antibody levels ten times higher than traditional vaccines, with ferritin nanoparticles significantly boosting immune responses (25). In terms of production and cost-effectiveness, ongoing research aims to optimize vaccine manufacturing processes to improve scalability and reduce costs. This is particularly crucial for low- and middle-income countries, which require affordable and effective vaccines. Overall, the future of vaccine research holds great potential for improving immunogenicity, safety, and accessibility. Continued innovation in carrier proteins, delivery methods, and production processes will be essential in addressing global health challenges and ensuring that vaccines remain a cornerstone of public health initiatives.

Serotype	Group	Statistics	Significance
1	1	0.959	0.799
	2	0.93	0.514
3	1	0.938	0.587
	2	0.93	0.518
4	1	0.944	0.651
	2	0.986	0.985
5	1	0.807	0.034
	2	0.832	0.063
6A	1	0.826	0.054
	2	0.914	0.383
6B	1	0.854	0.105
	2	0.89	0.236
7F	1	0.848	0.092
	2	0.851	0.097
9V	1	0.947	0.683
	2	0.907	0.332
14	1	0.895	0.261
	2	0.825	0.052
18C	1	0.911	0.36
	2	0.98	0.961
19A	1	0.855	0.107
	2	0.936	0.572
19F	1	0.972	0.915
	2	0.975	0.932
23F	1	0.833	0.064
	2	0.938	0.589

Table 4. Normal distribution test of vaccinated groups. Results of the Shapiro-Wilk test for the normal distribution of GMT values in both the Minhai (group 1) and Pfizer (group 2) groups for each serotype. Note: The degrees of freedom for all tests were 8.

MATERIALS AND METHODS

Immunization protocol

The vaccines used in the experiment included the Minhai 13 - valent pneumococcal polysaccharide conjugate vaccine, produced by Beijing Minhai Biotechnology Co., Ltd. (Approval number: 202204011, valid until April 5, 2024), and an imported vaccine produced by Pfizer (Import registration number: s20160042, China drug electronic supervision code: 81895490223773146383, valid until October 2024). Mice underwent basic and booster immunization. During the basic immunization period, which lasts 1 - 3 weeks after the vaccine is injected, a large amount of unstable IgM antibodies is produced. After 1 - 2 weeks, booster immunization is performed to convert IgM into IgG and enhance antibody affinity (26).

In the experiment, 28- to 35-day-old mice were used. The experimental and control groups were subcutaneously injected with 0.1 ml of the Minhai and Pfizer 13 - valent pneumococcal vaccine diluent (the vaccine was diluted with normal saline at a ratio of 1:10) on days 0, 21, and 28, while the negative group was subcutaneously injected with 0.1 ml of normal saline on the same schedule (27). Mice body weight was monitored at baseline (day 0, before immunization) and

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Serotype	Minahi mean	Pfizer mean	t	p
1	8609.11	6539.07	1.015	0.288
3	3540.08	4119.56	0.594	0.562
4	10928.58	12650.97	2.069	0.580
6A	8415.67	11139.24	0.851	0.409
6B	2891.16	4485.37	1.66	0.119
7F	3220.53	3983.95	1.015	0.327
9V	9668.16	17081.71	0.926	0.370
14	2200.31	5144.88	1.85	0.860
18C	9614.09	10565.70	0.992	0.338
19A	10787.73	9934.75	0.795	0.440
19F	6704.77	7110.40	0.301	0.768
23F	2563.84	3804.71	1.453	0.168

Table 5. Independent sample t-test between the Minahi and Pfizer groups. Comparison of GMT values between the Minahi and Pfizer groups for each serotype. α=0.05 was used as the significance level.

seven days after the third immunization (day 35). Weight was measured for all individuals using an electronic balance (±0.01 g, Sartorius). The selection of experimental animals is crucial in the design of the pneumococcal immunity experimental model. Based on previous research, we used SPF-grade BALB/c mice, which are 99% genetically identical to humans, had not been infected with pathogens, and were 4-7 weeks old adult male mice that were not affected by hormones or environmental factors (28, 29). The mice were provided by Beijing Weitongli Experimental Animal Co., Ltd. (Rodent Institute protocol number for this specific experiment: HIS-14). All mice exhibited smooth coats, were active, and had a good diet. To meet the 3R (Replacement, Reduction, Refinement) principle of experimental animals and statistical requirements, the number of experimental mice was determined by the degree of freedom (E) estimation method of biological experiments (30), that is, E = total number of experimental animals - number of groups, with $10 \leq E \leq 20$ indicating a normal sample size. Referring to this standard, the number of mice in the experimental and control groups was set at eight. A total of 56 mice were used, with 8 mice each in the Pfizer and Minhai vaccination groups, and 40 mice assigned to the negative control group (no vaccine). Each mouse was numbered and labeled for identification.

Blood collection

All experimental mice were bled from their orbits three to seven days after the last vaccine injection. The serum was separated by centrifugation, packaged, and stored at -40°C. The sera of 40 mice in the negative group were mixed and used as negative controls in the experiment.

Preparation of coated plates

Thirteen pneumococcal polysaccharide solutions (Beijing Gray Pharmaceutical Technology Co. Ltd.) were injected into 96 - well high - adsorption enzyme plates at 100 µl per well. After incubation at 37°C for 5 hours, wells were washed five times with 200 µl of 1X PBS (phosphate-buffered saline). Each well was then blocked with 5% bovine serum albumin (Solaibao) for 5 hours and washed again five times with 1X

Serotype	Minhai	Pfizer	t	df	P
3	3540	4119	-0.594	14	0.562
4	10928	12650	-2.069	14	0.058
6B	2891	4485	-1.660	14	0.119
7F	3220	3983	-1.015	14	0.327
14	2200	5786	-1.850	14	0.086
18C	9614	10565	-0.992	14	0.338

Table 6. Comparison of GMT of serotype antibodies with CRM197 and DT as carrier proteins. GMT values of serotype antibodies in the Minhai and Pfizer groups, along with t-test results. The t-test was performed to compare GMT values between the two groups. Test setting α=0.05, degrees of freedom υ=14.

Serotype	Minhai	Pfizer	t	df	P
1	8609	6539	1.105	14	0.228
5	9000	5341	3.459	14	0.004
6A	8415	11139	-0.851	14	0.409
9V	9668	10733	-0.926	14	0.370
19A	10787	9934	0.795	14	0.440
19F	6704	7110	-0.301	14	0.768
23F	2563	3804	-1.453	14	0.168

Table 7. Comparison of GMT of serotype antibodies with CRM197 and TT as carrier proteins. GMT values of serotype antibodies in the Minhai and Pfizer groups, along with t-test results. The t-test was performed to compare GMT values between the two groups. Test setting α=0.05 degrees of freedom υ=14.

PBS. The prepared polysaccharide-coated plates were stored in a 4°C incubator for future use. CRM197 and DT-coated plates were prepared in the same manner.

Antibody detection

Serum samples were diluted in normal saline at ratios of 1:500, 1:1000, 1:2000, 1:4000, and 1:8000, and added to the coated plate at 100 µl per well. The plate was incubated at 37°C for 2 hours, then washed five times with 200 µl of 1X PBS per well. Next, 100 µl of labeled monoclonal antibody (secondary antibody) was added to each well and incubated at 37°C for 1 hour. The plate was washed five times with washing buffer, followed by the addition of 100 µl of color development solution, which was incubated for 15 - 30 minutes. Finally, 10 - 20 µl of 2N H_2SO_4 was added to terminate the color development reaction. For each serotype tested, negative controls were also performed, and the obtained values were used to calculate the cutoff value for antibody positivity.

Detection method

The OD value was measured using the ELISA method. The treated 96-well plate was placed in a microplate reader (Thermo Scientific), with the wavelength set at 450 nm, and the plate's OD value was read. In this experiment, we utilized an ELISA reader (Thermo Scientific) to measure the optical density values of the samples. The ELISA plates w13 - valentsourced from Thermo Scientific. For the preparation of the ELISA assay, an antigen coating solution, serum diluent, enzyme, standard goat anti-mouse secondary antibody, washing buffer, colorimetric reagent, and stop solution

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were all obtained from Solaibao. Additionally, the 13-valent pneumococcal polysaccharide was purchased from Beijing Gray Pharmaceutical Technology Co., Ltd.

Data processing

The database was established using Excel 2013 to process the sample OD values and obtain the geometric mean titer (GMT) of the antibodies. SPSS 13.0 software was used to perform normal distribution tests, independent sample t-tests, and - Whitney U test on the GMT values.

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