HIGH-TEMPERATURE RESISTANT COMPOSITE POLYSACCHARIDE SYSTEM ANIMAL VACCINE ADJUVANT

Field of the Invention

The present invention relates to the field of animal vaccine adjuvant technology, specifically a high-temperature resistant composite polysaccharide system animal vaccine adjuvant.

Background to the Invention

At present, animal vaccine adjuvants play a key role in improving the immune efficacy of vaccines. However, adjuvants in existing technologies, especially polysaccharide adjuvants, still have obvious shortcomings: firstly, their heat resistance is generally poor, and most adjuvants have insufficient stability under high temperature conditions, which are prone to degradation or aggregation, leading to a decrease in activity. This makes vaccines heavily dependent on cold chain transportation throughout the entire supply chain, increasing costs and losses; Secondly, the immune enhancement effect is limited, and existing adjuvants often struggle to efficiently activate both humoral and mucosal immunity simultaneously, and have insufficient ability to induce long-term immune protection; Furthermore, safety and biocompatibility need to be improved. Some adjuvants, such as saponins, have issues such as hemolytic activity, high local inflammatory response, and may even pose a risk of immune escape.

Statement of Invention

（1） Technical problems solved

In response to the shortcomings of existing technology, the present invention provides a high-temperature resistant composite polysaccharide system animal vaccine adjuvant, which has the advantages of excellent high-temperature stability, comprehensive immune enhancement effect, and high biological safety. It solves the problems of insufficient stability, single immune activation, and high incidence of side reactions caused by poor heat resistance of existing adjuvants.

（2） Technical solution

To achieve the above objectives, the present invention provides the following technical solution: a high-temperature resistant composite polysaccharide system animal vaccine adjuvant, the adjuvant and its weight ratio are: β - glucan 0.5% to 2%; Tremella fuciformis polysaccharide 0.5% to 1.5%; Radix Astragali polysaccharide 0.3% to 1%; Angelica polysaccharide 1% to 2%; trehalose 5% to 10%; lactoferrin 0.5% to 2%; vitamin C palmitate 0.1% to 0.5%; vitamin E succinate 0.1% to 0.5%; squalene 10% to 20%; surfactant 1% to 3%; stabilizers 1% to 2%, with the remaining component being phosphate buffer solution at pH 6.5-7.4.

Preferably, the composite polysaccharide system is composed of β-glucan, Tremella fuciformis polysaccharide, Radix Astragali polysaccharide, Angelica polysaccharide, and trehalose.

Preferably, the lactoferrin and the composite polysaccharide system form an immune regulatory system.

Preferably, the vitamin C palmitate and vitamin E succinate form an antioxidant system.

Preferably, the high-temperature resistant composite polysaccharide system animal vaccine adjuvant, comprises the following steps:

step 1: preparation of formula ingredients: -prepare the formula proportions of β-glucan, Tremella fuciformis polysaccharide, Radix Astragali polysaccharide, Angelica polysaccharide, trehalose, lactoferrin, vitamin C palmitate, vitamin E succinate, squalene, surfactant, stabilizer, and phosphate buffer solution;

step 2: construction of composite polysaccharide system and antioxidant system: trehalose, vitamin C palmitate, β-glucan, Tremella fuciformis polysaccharide, Radix Astragali polysaccharide, Angelica polysaccharide, and lactoferrin are dissolved and assembled step by step to form a polysaccharide protein antioxidant composite aqueous phase;

step 3: preparation of oil phase emulsification system: mix squalene, vitamin E succinate, and surfactants by heating to prepare homogeneous oil phase colostrum containing fat soluble antioxidants;

step 4. high pressure homogenization and stabilization treatment: the colostrum and stabilizer obtained in step 3 are homogenized under high pressure to refine the droplet size of the emulsion, and combined with the stabilizer to build a three-dimensional protection network to obtain a homogenized adjuvant lotion;

step 5: terminal aseptic treatment and subpackaging: the homogenized adjuvant lotion obtained in step 4 is passed through terminal sterilization and subpackaging process to extend the validity period of normal temperature storage.

Preferably, the construction of the composite polysaccharide system and antioxidant system:

S1.1. dissolve trehalose and vitamin C palmitate in phosphate buffer solution at 60-65 ℃, stir at 500-550rpm for 8-10 minutes until completely clear, and use as the base aqueous phase;

S1.2. under high-speed shearing at 8000-10000rpm, β-glucan, Tremella fuciformis polysaccharide, Radix Astragali polysaccharide, and Angelica polysaccharide are sequentially added to the base aqueous phase at a rate of 0.5-1.0g/min, and the system temperature is controlled to ≤ 70 ℃ until colloids are formed;

S1.3. slowly disperse lactoferrin in the above colloid in a water bath at 38-40 ℃, and stir at low speed of 300-350rpm for 25-30 minutes to obtain polysaccharide protein nanocomposites.

Preferably, in step 3, the preparation process of the oil phase emulsification system in step 3:

S2.1. mix squalene, vitamin E succinate, and surfactant at 55-60 ℃ and stir at 400-450rpm until an oil phase is formed;

S2.2. under high-speed shearing at 18000-20000 rpm, add the oil phase prepared in step three dropwise to the aqueous phase of the composite polysaccharide system obtained in step two, and continue shearing for 5-8 minutes to form colostrum.

Preferably, in step 4, high-pressure homogenization and stabilization treatment:

S3.1. the obtained colostrum is homogenized for 3 to 5 times under 100-150 MPa pressure through a high-pressure homogenizer at 2-8 ℃ until the particle size of lotion PDI is less than 0.15;

S3.2. add stabilizer to the homogenized lotion, and mix it for 25-30 minutes at a low speed of 200-250 rpm under the protection of nitrogen.

Preferably, the terminal aseptic treatment in step 5: the final adjuvant lotion is aseptic filtered through 0.1 μ m or 0.22 μ m microporous membrane.

Preferably, in step 5, the packaging process: in a sterile filling line under laminar flow

protection of Class A cleanliness, penicillin bottles and butyl rubber stoppers pre sterilized by dry heat at 170-180 ℃ for 1.8-2 hours are used for packaging, and nitrogen gas with a purity of ≥ 99.999% is immediately filled to replace the headspace oxygen and sealed, and stored in the dark at 2-8 ℃.

Compared with the existing technology, the present invention provides a high-temperature resistant composite polysaccharide system animal vaccine adjuvant, which has the following beneficial effects:

1. The present invention constructs a composite polysaccharide system composed of β-glucan, Tremella fuciformis polysaccharide, Radix Astragali polysaccharide, Angelica polysaccharide, and trehalose, and synergistically forms an immune regulatory network with lactoferrin, achieving significant enhancement of adjuvant immune activation ability, synchronous enhancement of humoral and mucosal immunity, and effective reduction of inflammatory side effects.

2. The present invention uses trehalose as the core heat-resistant protective agent, combined with a composite antioxidant system composed of vitamin C palmitate and vitamin E succinate, to achieve long-term maintenance of adjuvant structure and functional integrity under high temperature conditions (storage at 60℃ for 3 months with activity retention rate≥90%), thereby greatly improving the beneficial effects of product storage stability and shelf life.

3. By optimizing the oil phase emulsification process and high-pressure homogenization technology, combined with the stabilizer, the invention constructs a nanometer level uniform lotion system, which achieves the beneficial effects of improving the adjuvant biocompatibility (hemolysis index≤3.3%, side reaction rate≤3.5%), completely eliminating the risk of immune escape, and ensuring controllable production process and high stability

between batches.

Brief Description of the Drawings

Figure 1 is a flowchart of the adjuvant preparation process of the present invention.

Detailed Description

Below, the technical solutions in the embodiments of the present invention will be clearly and completely described in conjunction with the accompanying drawings. Obviously, the described embodiments are only a part of the embodiments of the present invention, not all of them. Based on the embodiments of the present invention, all other embodiments obtained by ordinary skilled persons in the art without creative labor are within the scope of protection of the present invention.

Please refer to Figure 1, a high-temperature resistant composite polysaccharide system animal vaccine adjuvant, the adjuvant and its weight ratio are: β-glucan 0.5% to 2%; tremella fuciformis polysaccharide 0.5% to 1.5%; Radix Astragali polysaccharide 0.3% to 1%; Angelica polysaccharide 1% to 2%; trehalose 5% to 10%; lactoferrin 0.5% to 2%; vitamin C palmitate 0.1% to 0.5%; vitamin E succinate 0.1% to 0.5%; squalene 10% to 20%; surfactant 1% to 3%; stabilizers 1% to 2%, with the remaining component being phosphate buffer solution at pH 6.5-7.4 are supplemented to 100%.

Specifically, the raw materials and their functions are:

Table 1

Material Name	Weight ratio range	Effect

β-glucan	0.5%～2%	The core component of the composite polysaccharide system activates macrophages/DC cells, enhances cell-mediated immunity, and the triple helix conformation ensures high temperature stability
Tremella fuciformis polysaccharide	0.5%～1.5%	Compound polysaccharide system components to form a three-dimensional gel network, synergistically enhance mucosal immunity and reduce local side effects
Radix Astragali polysaccharide	0.3%～1%	Compound polysaccharide system components promote B cell proliferation and antibody production, eliminate free radicals, and inhibit the release of inflammatory factors
Angelica polysaccharide	1%～2%	Compound polysaccharide system components enhance NK cell killing activity, inhibit excessive immune response, and reduce hemolysis risk
Trehalose	5%～10%	Composite polysaccharide system components+stabilizers form a hydrogen bond network to protect polysaccharide conformation and enhance high-temperature storage stability
Lactoferrin	0.5%～2%	The core of the immune regulatory system blocks pathogen iron uptake, enhances antigen presentation, and reduces the risk of immune escape
Vitamin C palmitate	0.1%～0.5%	Antioxidant system components, fat soluble antioxidant, protect polysaccharides/proteins from oxidative degradation
Vitamin E succinate	0.1%～0.5%	Antioxidant system components, water-soluble antioxidant, synergistically enhance with vitamin C palmitate, regenerate vitamin E
Squalene	10%～20%	Oil phase carrier, forming water in oil emulsion to prolong

		antigen retention time and enhance immune memory
Surfactant	1%～3%	Preferred combination of polysorbate 80 and polyglycerol fatty acid ester (2:1) to enhance oil-water compatibility and stabilize emulsion particle size
Stabilizer	1%～2%	Preferred hydroxypropyl - β - cyclodextrin and sodium glycerophosphate (1:1) to inhibit particle aggregation and maintain system osmotic pressure stability
Phosphate buffer solution （PBS）	Margin	Solvent, maintain pH 6.5-7.4, ensure compatibility and biological activity of each component

Specifically, β-glucan, Tremella fuciformis polysaccharide, Radix Astragali polysaccharide, Angelica polysaccharide, and trehalose form a complex polysaccharide system;

β-glucan is yeast derived β-1,3/1,6-glucan, with a molecular weight of 50-200kDa, triple helix conformation integrity ≥ 95%, drying loss ≤ 5.0%, ignition residue ≤ 1.5%, bacterial endotoxin<0.5EU/mg, glass transition temperature (Tg) ≥ 120 ℃, and viscosity retention rate ≥ 85% after 30 minutes of heat treatment at 100 ℃;

Tremella fuciformis polysaccharide is an acid heteropolysaccharide of plant origin (glucuronic acid content ≥ 25%), with a molecular weight of 80-300kDa. There is a characteristic peak of β-glycoside bond in the infrared spectrum at 890cm^{-1}. A three-dimensional reticular gel is formed in an aqueous solution at pH6.0-7.5, and the dynamic light scattering particle size distribution PDI is less than 0.2;

Radix Astragali polysaccharides are extracted from the root of Astragalus membranaceus, with a molar ratio of mannose: glucose of 3:1, UV260/280 ≥ 1.8 (no nucleic acid/protein contamination), total sugar content ≥ 90%, free radical scavenging rate (DPPH method) ≥

70%, and can activate macrophages to increase NO secretion by 3.2 times;

Angelica polysaccharide is a ferulic acid bound polysaccharide (with a phenolic hydroxyl content of ≥ 8%), with a molecular weight of 100-400 kDa and an HPLC fingerprint matching degree of ≥ 0.95. It inhibits LPS induced IL-6 release with an IC_{50} of 12.5 μ g/mL and a hemolysis index of<5% (rabbit red blood cell method);

Trehalose is a disaccharide crystal (CAS 99-20-7) with a content of ≥ 99.0%, moisture content≤0.5%, melting point 203-206℃, and glass transition temperature (Tg) of the aqueous solution reaching 110℃. The ice crystal sublimation rate during freeze-drying is controlled at 0.8g H_2O/(g · h) to maintain the polysaccharide network structure.

Specifically, lactoferrin and complex polysaccharide system form an immune regulatory system. Lactoferrin is a human or bovine milk derived iron binding glycoprotein with iron saturation ≤ 15%, molecular weight 76-80 kDa, isoelectric point (pI) 8.0-8.5, HPLC purity ≥ 98.0%, microbial limit ≤ 100 CFU/g, heavy metals (calculated as Pb) ≤ 10 ppm, and a blocking efficiency of pathogen iron uptake>90%.

Specifically, vitamin C palmitate and vitamin E succinate form an antioxidant system;

Vitamin C palmitate is a white crystalline powder (CAS 137-66-6) with a content of ≥ 98.0%, a melting point of 105-108℃, an oil-water partition coefficient logP=6.2 ± 0.3, a DPPH radical scavenging rate EC_{50}=8.2 μM, and a half-life of ≥ 96 hours in pH 4.0-7.0 buffer solution;

Vitamin E succinate is a light yellow oily substance (CAS 118-62-5), with an α- tocopherol equivalent of ≥ 90.0%, an acid value of ≤ 2.0mg KOH/g, a peroxide value of ≤ 5.0meq/kg, a regeneration efficiency of 85% for vitamin E, and a hydrogen bond binding energy with polysaccharides of ≥ 25kJ/mol.

Specifically, a high-temperature resistant composite polysaccharide system animal vaccine adjuvant comprising the following steps:

Step 1: preparation of formula ingredients: -prepare the formula proportions of β-glucan, Tremella fuciformis polysaccharide, Radix Astragali polysaccharide, Angelica polysaccharide, trehalose, lactoferrin, vitamin C palmitate, vitamin E succinate, squalene, surfactant, stabilizer, and phosphate buffer solution;

Step 2: construction of composite polysaccharide system and antioxidant system: trehalose, vitamin C palmitate, β-glucan, Tremella fuciformis polysaccharide, Radix Astragali polysaccharide, Angelica polysaccharide, and lactoferrin are dissolved and assembled step by step to form a polysaccharide protein antioxidant composite aqueous phase; The core guarantee is the integrity of the triple helix conformation of the complex polysaccharide system and the retention of lactoferrin activity;

Step 3: preparation of oil phase emulsification system: mix squalene, vitamin E succinate, and surfactants by heating to prepare homogeneous oil phase colostrum containing fat soluble antioxidants; And prepare for subsequent high-energy emulsification;

Step 4. high pressure homogenization and stabilization treatment: the colostrum and stabilizer obtained in step 3 are homogenized under high pressure to refine the droplet size of the emulsion, and combined with the stabilizer to build a three-dimensional protection network to obtain a homogenized adjuvant lotion, and improve the high-temperature stability and storage period of adjuvants;

Step 5: terminal aseptic treatment and subpackaging: the homogenized adjuvant lotion obtained in step 4 is passed through terminal sterilization and subpackaging process, ensure the sterility and safety of adjuvants,and extend the validity period of normal temperature storage.

Specifically, in step 2, the construction of the composite polysaccharide system and antioxidant system:

S1.1. Dissolve trehalose and vitamin C palmitate in phosphate buffer solution at 60-65 ℃, stir at 500-550rpm for 8-10 minutes until completely clear, and use as the base aqueous phase;

S1.2. Under high-speed shearing at 8000-10000rpm, β-glucan, Tremella fuciformis polysaccharide, Radix Astragali polysaccharide, and Angelica polysaccharide are sequentially added to the base aqueous phase at a rate of 0.5-1.0g/min, and the system temperature is controlled to ≤ 70℃ until a uniform, semi transparent viscous colloid is formed;

S1.3. The lactoferrin was slowly dispersed in the above colloid in a water bath at 38-40 ℃, and stirred at a low speed of 300-350 rpm for 25-30 minutes to avoid excessive foam and complete the pre assembly of polysaccharide protein nanocomposites.

Through the above steps, the directed binding of the composite polysaccharide system with antioxidants and lactoferrin is achieved, with a polysaccharide triple helix conformation retention rate of ≥ 95% and lactoferrin activity loss of ≤ 5%, resulting in no stratification or precipitation of the composite aqueous phase after 72 hours of storage at 70℃.

Specifically, the preparation process of the oil phase emulsification system in step three:

S2.1. Mix squalene, vitamin E succinate, and surfactant at 55-60 ℃ and stir at 400-450rpm until a homogeneous oil phase is formed;

S2.2. Slowly add the oil phase prepared in step three to the aqueous phase of the composite polysaccharide system obtained in step two under high-speed shearing at 18000-20000 rpm, and continue shearing for 5-8 minutes to form colostrum.

Through the above steps, water in oil colostrum with oil droplet size ≤ 2 μ m is obtained. Vitamin E succinate is uniformly dispersed in the oil phase and forms a complete antioxidant network with vitamin C palmitate in the water phase, resulting in a 24-hour stability of ≥ 98% for the colostrum.

Specifically, in step four, high-pressure homogenization and stabilization treatment:

S3.1. The obtained colostrum is homogenized for 3 to 5 times under 100-150 MPa pressure through a high-pressure homogenizer at 2-8 ℃ until the particle size of lotion PDI is less than 0.15;

S3.2. Add stabilizer to the homogenized lotion, and mix it for 25-30 minutes at a low speed of 200-250 rpm under the protection of nitrogen.

Through the above steps, the average particle size of the emulsion drop is controlled at 100-300nm, and the PDI is ≤ 0.15. The stabilizer forms a dense protective shell on the surface of the emulsion drop to inhibit the aggregation of particles at high temperature, so that the homogeneous solution can be stored for 3 months at 60 ℃, and the change rate of lotion particle size is ≤ 5%.

Specifically, the terminal aseptic treatment in step 5: sterilize the final adjuvant lotion through 0.1 μ m or 0.22 μ m microporous membrane.

Specifically, in step five, the packaging process involves using penicillin bottles and butyl rubber stoppers that have been pre sterilized by dry heat at 170-180 ℃ for 1.8-2 hours on a sterile filling line under laminar flow protection of Class A cleanliness. The bottles are then immediately filled with nitrogen gas with a purity of ≥ 99.999% to replace the headspace oxygen, sealed, and stored in the dark at 2-8 ℃.

Through the above steps, the sterile qualification rate of the adjuvant is 100%, the

headspace oxygen content after nitrogen sealing is ≤ 0.5%, and the shelf life of the finished adjuvant is ≥ 18 months when stored at room temperature of 25 ℃, and ≥ 90% when stored at high temperature of 60 ℃ for 3 months.

(1)A certain experiment will implement the above adjuvant formula, as follows:

Embodiment 1

β-glucan 1.0%; Tremella fuciformis polysaccharide 1.0%; Radix Astragali polysaccharide 0.6%; Angelica polysaccharide 1.5%; Trehalose 8%; Lactoferrin 1.2%; Vitamin C palmitate 0.3%; Vitamin E succinate 0.3%; Squalene 15%; Surfactant 2%; Stabilizer 1.5%; Supplement PBS to 100% (pH 7.0).

Embodiment 2

β-glucan 0.5%; Tremella fuciformis polysaccharide 1.5%; Radix Astragali polysaccharide 0.3%; Angelica polysaccharide 1.0%; Trehalose 5%; Lactoferrin 0.5%; Vitamin C palmitate 0.1%; Vitamin E succinate 0.1%; Squalene 10%; 1% surfactant; Stabilizer 1%; Supplement PBS to 100% (pH 6.5).

Embodiment 3

β-glucan 2.0%; Tremella fuciformis polysaccharide 0.5%; Radix Astragali polysaccharide 1.0%; Angelica polysaccharide 2.0%; Trehalose 10%; Lactoferrin 2.0%; Vitamin C palmitate 0.5%; Vitamin E succinate 0.5%; Squalene 20%; Surfactant 3%; Stabilizer 2%; Supplement PBS to 100% (pH 7.4).

Embodiment 4

β-glucan 1.5%; Tremella fuciformis polysaccharide 1.2%; Radix Astragali polysaccharide 0.8%; Angelica polysaccharide 1.8%; Trehalose 7%; Lactoferrin 1.5%; Vitamin C palmitate 0.4%; Vitamin E succinate 0.4%; Squalene 18%; Surfactant 2.5%; Stabilizer 1.8%;

Supplement PBS to 100% (pH 7.2).

(2)Take the comparative example as the reference group for the embodiment, as follows:

Comparative example 1

The absence of Angelica polysaccharides in the complex polysaccharide system, and the remaining components and ratios are the same as in Embodiment 1 (to verify the anti-inflammatory and immunomodulatory effects of Angelica sinensis polysaccharides).

Comparative example 2

The antioxidant system (vitamin C palmitate+vitamin E succinate) is missing, and the remaining components and ratios are the same as in Embodiment 2 (to verify the high-temperature protective effect of the antioxidant system).

Comparative example 3

Replace lactoferrin with traditional saponin adjuvant (Quil A), and the remaining components and ratios are the same as in Embodiment 3 (to verify the safety of the immune regulatory system).

Comparative example 4

Replace trehalose with regular sucrose, and the remaining components and ratios are the same as in Embodiment 4 (to verify the high-temperature stability of trehalose).

The performance tests of the prepared finished adjuvants and commercially available adjuvants were conducted based on the embodiments and comparative examples. The test data are shown in Table 2:

Table 2

Sample Type	Activity retention rate after 3 months of storage at 60 ℃	Stability after 18 months of storage at 25 ℃	Immune enhancement rate (antibody titer multiple)	Mucosal immune activation rate (sIgA level)	Incidence of adverse reactions after vaccination (fever/swelling)	Hemolysis index (%)	Immune escape risk (in vitro experiment)
Embodiment 1	92%	Qualified	8.6 times	78%	3.2%	3.1%	No
Embodiment 2	90%	Qualified	7.2 times	72%	2.8%	2.8%	No
Embodiment 3	93%	Qualified	9.3 times	82%	3.5%	3.3%	No
Embodiment 4	91%	Qualified	8.9 times	80%	3.0%	3.0%	No
Comparative example 1	85%	Qualified	6.1 times	63%	8.5%	4.8%	No
Comparative example 2	72%	Unqualified	5.8 times	59%	7.9%	4.5%	No
Comparative	88%	Qualified	7.5 times	68%	15.8%	18.6%	Yes

example 3							
Comparative example4	65%	Unqualified	4.2 times	51%	6.7%	4.2%	No
Commercial adjuvant (comparative group)	60%	Unqualified	5.5 times	55%	12.5%	10.2%	Low risk

Note: ① The activity retention rate was detected by ELISA to assess the immune enhancing activity of adjuvants on vaccine antigens; ② Stability criteria: no stratification, no precipitation, particle size change rate ≤ 10%; ③ The immune enhancement rate is the ratio of the antibody titer in animal serum after vaccination to the control group (adjuvant free vaccine); ④ The incidence of adverse reactions is the statistical result of adverse reactions within 72 hours after vaccination in 100 experimental animals (mice); ⑤ The hemolysis index is detected using the rabbit red blood cell method, with a safety standard of ≤ 5%; ⑥ The risk of immune evasion is detected through in vitro cell culture experiments, and the judgment criteria are whether it promotes the pathogen to evade recognition and clearance by host immune cells.

According to Table 2:

(1)The high-temperature resistant composite polysaccharide system adjuvants prepared in Comparative examples 1-4 showed significant advantages in various key performance indicators. In terms of stability, the prepared adjuvants had activity retention rates of over

90% after accelerated storage at 60 ℃ for 3 months, and their physical properties stabilized after long-term storage at 25 ℃ for 18 months, demonstrating their excellent high-temperature resistance and long-term storage stability; In terms of immune efficacy, the adjuvant immune enhancement rate (antibody potency multiple) is as high as 7.2-9.3 times, and the mucosal immune activation rate (sIgA level) reaches 72% -82%, far superior to the reference group, indicating that the adjuvant can efficiently stimulate strong humoral and mucosal immunity simultaneously; In terms of safety, the incidence of side reactions (2.8% to 3.5%) and hemolysis index (2.8% to 3.3%) after vaccination are at extremely low levels, and there is no risk of immune escape, demonstrating its excellent biocompatibility and reliability.

(2) Due to the absence or replacement of some components in the Comparative examples 1-4, significant performance shortcomings were observed. In comparative example 1, due to the absence of Angelica sinensis polysaccharides, the immune enhancement rate, mucosal immunity, and side reaction control were inferior to those in embodiment 1, confirming that the Angelica polysaccharides of the present invention can synergistically regulate immunity and alleviate inflammatory responses; Due to the lack of an antioxidant system, the retention rate and stability of the prepared adjuvant in Comparative Example 2 were severely reduced, demonstrating that the composite antioxidant system composed of vitamin C palmitate and vitamin E succinate in the present invention can maintain the activity of the adjuvant at high temperatures; Due to the replacement of lactoferrin with Quil A in Comparative Example 3, although the immune effect is still acceptable, the incidence of side reactions and hemolysis index sharply increase, and there is a risk of immune escape, highlighting that the lactoferrin immune regulatory system of the present invention can balance efficiency and safety; Due to the substitution of trehalose with sucrose in Comparative Example 4, the heat resistance and immune efficacy of the prepared adjuvant

significantly deteriorated, directly proving that trehalose is the core heat-resistant protective agent in the present invention.

(3) As a control group, commercially available adjuvants lag behind various embodiments of the present invention in terms of activity retention rate, stability, immune efficacy, and safety, particularly in terms of poor stability, high side reaction rate, and potential safety risks. This further validates the innovation of the technical solution of the present invention.

Summary: The present invention successfully provides a composite polysaccharide system animal vaccine adjuvant that combines excellent high temperature stability, strong bidirectional immune activation ability (humoral and mucosal immunity), and extremely high biological safety. Through the scientific combination and synergistic effect of the composite polysaccharide system, immune regulation system (lactoferrin), antioxidant system, and heat-resistant protection system (trehalose), and relying on optimized preparation processes, it systematically solves the industry technical bottlenecks of poor stability, single immune effect, and high side reaction rate commonly found in existing adjuvants. The experimental data fully confirms that the performance of the adjuvant of the present invention is superior to traditional commercially available products, providing key technical and material support for the development of a new generation of efficient, safe, and stable animal vaccines.

Although the embodiments of the present invention have been shown and described, it will be understood by those skilled in the art that various changes, modifications, substitutions, and variations can be made to these embodiments without departing from the principles and spirit of the present invention. The scope of the present invention is limited by the appended claims and their equivalents.