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Comparative efficacy of different Carbopol concentrations as a stabilizer of live attenuated sheep pox virus vaccine against lumpy skin disease

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In Egypt, the lyophilized live attenuated sheep pox virus vaccine has been used for the vaccination of cattle against lumpy skin disease virus to control its economic impact on livestock industry. In this endeavor, we validate the efficacy of Carbopol® as a stabilizer and adjuvant to enhance immunogenicity of such a heterologous sheep pox virus vaccine against lumpy skin disease. Lyophilization of sheep pox virus vaccine stabilized with Carbopol® produced better physical and antigenic properties than freeze-drying with lactalbumin/sucrose stabilizer; this was manifested by superior disc uniformity, thermo-stability at 37°C, and less reduction in virus titer. Immunization of calves' groups with variable sheep pox vaccine doses containing different Carbopol® concentrations revealed that 10^{3.5} TCID₅₀ of sheep pox virus vaccine enclosing 0.5% Carbopol® is the field dose of choice. Moreover, it induced protective serum neutralizing index of 2.5 and a ELISA S/P ratio of 36, by the 4th week post vaccination. Besides, the inclusion of 0.5% Carbopol® in formulation of the sheep pox virus vaccine was safe in bovines and enhanced cellular immune response to lumpy skin disease virus, as evidenced by increased T cell proliferation. Hence, it is recommended to use Carbopol® as 0.5% in preparation of live attenuated sheep pox virus vaccine to confer better protection against lumpy skin disease virus infection.

Keywords: lumpy skin disease; sheep pox virus; vaccination; cellular immune response; ELISA.

Introduction

Lumpy skin disease (LSD) is an acute infectious disease of cattle and has become endemic in Egypt and other African and Asian countries, exerting a serious economic impact on livestock industry. It is caused by LSD virus, a member of the *Capripoxvirus* genus and the *Poxviridae* family, along with the caprine pox virus (GPV) and the ovine pox virus (SPV).^(1,2) In Egypt, LSD was reported in 1988, at the Suez quarantine station and reappeared during 2005, 2006, 2012, 2013 and 2018, with significant economic losses.⁽³⁾ Clinical signs include fever, cutaneous nodules on different regions of animal body and lesions in mucous membranes of the eyes, mouth and nose. Additionally, LSD causes reduced milk production, weight loss, abortions, inconsumable meat and skin, and death, especially in young calves.⁽³⁾

LSD virus (LSDV), SPV and GPV are closely antigenically and genetically related, share 97% of genomic nucleotide sequence identity and induce a reasonable cross-protection.⁽⁴⁾ Therefore, use of a capri pox virus vaccine strain (for example, SPV vaccine) can protect cattle against LSD and sheep and goats against sheep pox virus and goat pox virus diseases, respectively.⁽⁵⁾ In Egypt (unlike other African countries that have used the homologous Neethling LSD strain vaccine), the Kenyan SPV vaccine was used during 2005-2006. Currently, the Romanian SPV strain vaccine is used to immunize cattle against LSD. It provides a partial tackle for LSD outbreaks with partial cross protection and short-lived immunity. After the recent LSD outbreaks, the use of the SPV vaccine has shown inadequate protection against LSD in cattle.⁽⁶⁾

The development of effective vaccines is an approach to provide cost-effective intervention against zoonotic

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and animal infectious diseases. In regions with a given enzootic disease, vaccination of the susceptible animal host is the milestone to control such a disease. Vaccines, whether live or inactivated, require formulations with adjuvants that act as vehicles or immunostimulants. Carbomers are synthetic, high molecular weight, nonlinear polymers of acrylic acid, cross-linked with a polyalkenyl polyether. These polymers are safely used in pharmaceutical and cosmetic preparations as thickening, suspending, dispersing, emulsifying or stabilizing mediators with different concentrations (0.1% - 50%), mostly below 1%.^(7,8)

Carbomers have been used as adjuvants in veterinary vaccines since 1970s, as they facilitate vaccine delivery and enhance cellular immune response with prolonged duration whether live or inactivated, such as: live-attenuated Newcastle disease virus (NDV) vaccine; modified-live vaccine for porcine reproductive and respiratory syndrome (PRRS1); swine parvovirus vaccine; circovirus type 2 vaccine; *Staphylococcus aureus* vaccine for sheep; freeze-dried inactivated bovine viral respiratory combined vaccine; inactivated equine herpes virus-1 vaccine; inactivated rabies vaccine and freeze-dried bovine ephemeral fever virus vaccine.^(9,10,11,12,13) These studies proved the safe use of carbomers in vaccine preparation and stronger immune responses than those obtained by traditional vaccines. Carbopol® enhances cellular immunity by targeting a strong type-1 T-cell (Th1) polarization and induction of interferon-gamma (IFN γ) production. It also enhances antigen capture by macrophages, especially the dendritic cells.^(14,15)

This study attempted to evaluate Carbomers to improve the stability of locally produced Romanian SPV vaccine and to enhance its protective efficacy as a heterologous vaccine against LSD infection in cattle.

Materials and Methods

Ethical approval

This study was approved by the Animal Ethics Committee of the Veterinary Sera and Vaccines Research Institute (VSVRI). All experiments matched with the VSVRI guidelines for animal research.

Cell culture and virus

African green monkey Kidney (Vero) cells were grown at 37°C in Minimum Essential Medium with Earle's salts (MEM, Sigma Chemical Company, UK) supplemented

with heat-inactivated 10% Newborn calf serum, 100 UI/mL penicillin, 100 μ g/mL streptomycin sulphate and 25 IU/L mycostatin (Gibco Laboratories, New Zealand).

The live attenuated sheep pox virus (SPV, Romanian strain), with a titer of $10^{5.5}$ TCID₅₀/mL, was kindly obtained from the Pox Vaccines Researches Department (PVRD), VSVRI, Abbassia, Cairo, Egypt.

The virus was propagated and titrated on Vero cell line which has been proved free of any extraneous contamination. Both virus and cell culture were used in vaccine preparations and virus neutralization test (VNT).⁽¹⁾

Titration of SPV vaccine on Vero cell line

The prepared live SPV vaccines using Carbopol® and lactalbumin hydrolysate/sucrose was titrated on Vero cell line.⁽¹⁾

Control sera

Positive anti-SPV serum

Anti-SPV serum was prepared in three adult New Zealand rabbits (2-3 kg body weight), for use as a positive control serum in the VNT.⁽¹⁾

Negative serum

Antibody free Newborn calf serum (Gibco Laboratories, New Zealand) was used as negative control serum in VNT.

Preparation of carbomers

The carbomer used in vaccine preparations was Carbopol® 940 NF polymer (Lubrizol®). It was dissolved in hot double-distilled water at final concentrations of 0.25%, 0.5% and 1%, then solutions were sterilized separately by autoclaving at 121°C for 20 min and stored at 4°C. Before use in vaccine preparation, all Carbopol® solutions were adjusted to pH 7.3.

Calves and vaccination

Thirty susceptible native breed calves 4-12 months old were assigned into nine groups, three calves each. They were proved free from virus neutralizing antibody against LSDV by VNT using SPV.

All animals were housed in hygienic, insect proof isolated units, at VSVRI animal facility, with daily clinical observation for 2 weeks before and during the experimentation period. Heparinized whole blood and serum samples were collected post vaccination (PV) at

pre-scheduled intervals. Sera were stored at -20°C until testing by ELISA and VNT to evaluate the immune response to the prepared SPV vaccine formulations.

Vaccine formulations

Sheep pox virus, titer $10^{5.5}$ TCID₅₀/mL, was inoculated into Vero cell line and harvested after 4 days post inoculation when monolayers showed 70% cytopathic effect. After three freeze-thaw cycles, centrifugation was carried out and the supernatant was collected. Then two freeze-dried SPV vaccine formulations were prepared using different stabilizers as follows:

- Formula 1 was stabilized by the ordinary stabilizer consisting of a 5% lactalbumin hydrolysate (Sigma-Aldrich GmbH) with a 2.5% sucrose (Difco Laboratories, USA).⁽¹⁾
- Formulas 2, 3 and 4 were stabilized by 0.25%, 0.5% and 1% carbomers (Carbopol® 940 NF, Lubrizol, USA), respectively.^(10,11,13)

Quality control testing of the prepared SPV vaccine formulations

Physical appearance

All lyophilized SPV vaccine formulations containing Carbopol® and lactalbumin hydrolysate/sucrose stabilizer underwent physical examination.

Thermo stability test

Samples of the prepared SPV vaccine, stabilized with the Carbopol® concentration, selected as the best, were kept at 37°C for one week and subjected to virus titration every 24 hour.⁽¹⁶⁾

Sterility

Random samples from the prepared SPV vaccines stabilized with Carbopol® were tested for their freedom from aerobic and anaerobic bacteria, fungi and mycoplasma on thioglycolate medium, Sabouraud agar, nutrient agar and mycoplasma medium.⁽¹⁾

Safety

A group of three susceptible calves (Group 10) were vaccinated with 10X Carbopol® stabilized SPV vaccine field dose.⁽¹⁾

Calves immunization with carbomers-stabilized SPV vaccines

Calves (groups 1–4) were vaccinated with the prepared Carbopol®-stabilized SPV vaccine formulations, as follows:

Group 1: vaccinated with SPV vaccine stabilized with 0.25% Carbopol®.

Group 2: vaccinated with SPV vaccine stabilized with 0.5% Carbopol®.

Group 3: vaccinated with SPV vaccine stabilized with 1% Carbopol®.

Group 4: vaccinated with SPV vaccine stabilized with 5% lactalbumin hydrolysate/2.5% sucrose.

Group 5: non-vaccinated control.

Serum samples were taken weekly post vaccination up to 6 weeks for VNT and ELISA from all calves' groups.

Evaluation of humoral immune response

Virus neutralization test

VNT was conducted to estimate the seroconversion (antibody titers) in sera from vaccinated calves, as well as to determine the virus neutralization indices (NI) which were calculated by the following equation: NI = Virus Titer (VT)- Serum Virus Titer (SVT), where NI >1.5 were consider positive results.^(1,6)

Commercial ELISA kit

A commercial ELISA kit ID Screen® Capripox Double Antigen Multi-species, manufactured by IDvet (France) ID Vet., batch /N*de lot H35, was used to screen serum samples collected from the different calves' groups. The ELISA test was done according to manufacturer's instructions. The results were interpreted based on the calculated S/P ratio.

$$S/P = \frac{\text{test sample OD} - \text{negative control OD}}{\text{positive control OD} - \text{negative control OD}} * 100$$

If the S/P ratio is higher than 30%, the tested sample is considered a positive result for the presence of LSDV specific antibodies.

Cell proliferation assay

Collected heparinized blood samples at days 3, 5, 7, 10, 14, 21 and 28 post vaccination from all vaccinated and non-vaccinated calves, were used for the cell proliferation assay that was performed using the cell

proliferation kit (XTT, Cat. No.11465017005, Sigma-Aldrich). Optical density (OD) was measured using an ELISA reader.

Determination of the optimal field dose of SPV vaccines

The optimum field protective dose of SPV vaccine was verified by vaccinating calves (groups 6-9) intradermally, with different potential doses, as shown hereafter:

Group 6: vaccinated with a dose of $10^{3.5}$ TCID₅₀/animal of Carbopol® stabilized SPV vaccine.

Group 7: vaccinated with a dose of 10^4 TCID₅₀/animal of Carbopol® stabilized SPV vaccine.

Group 8: vaccinated with a dose of $10^{3.5}$ TCID₅₀/animal of lactalbumin hydrolysate/sucrose stabilized SPV vaccine.

Group 9: vaccinated with a dose of 10^4 TCID₅₀/animal of lactalbumin hydrolysate/sucrose stabilized SPV vaccine.

Serum samples were taken weekly post vaccination up to 6 weeks for VNT and ELISA from all calves' groups.

Results

Determination of the best concentration of Carbopol® added to SPV vaccines

The mean NI and S/P ratio obtained for the SPV vaccines stabilized with different concentrations of Carbopol® are tabulated in Table 1. Protective levels of antibodies were induced in all calves immunized with vaccines containing different concentrations of

Carbopol® (groups 1, 2 and 3), as well as in calves immunized with the lactalbumin hydrolysate/sucrose stabilized vaccine (group 4). Non-protective antibodies were induced in control calves (group 5). The highest levels of antibodies were detected in groups 2 and 3, which showed the highest NI value (3, for both groups) and similar S/P values (45%, 47% respectively); while in calves immunized with the lactalbumin hydrolysate/sucrose stabilized vaccine (group 4), the NI value was 2.50 and S/P=41%. Therefore, 0.5% Carbopol® was the concentration of choice for using with SPV vaccine ($10^{3.5}$ TCID₅₀/field dose).

Quality control of prepared live attenuated Carbopol® stabilized SPV vaccines

Physical appearance

As shown in Figure 1, the lyophilized vaccine prepared with lactalbumin hydrolysate/sucrose stabilizer alone was yellowish, uniform in shape, and somewhat adherent to the vial walls with some vacuolation in the vaccine disk, in contrast, the lyophilized vaccine formulations with 0.5% Carbopol® exhibited better physical properties, the disk remained situated within the walls of the vials as whitish uniform compact solid disks.

Titration of prepared SPV vaccines with different concentrations of Carbopol® before and after lyophilization

Stabilized SPV vaccines prepared with different concentrations of Carbopol® showed the same reduction in virus titer ($0.25 \log_{10}$ TCID₅₀/mL) after lyophilization,

Table 1. Distribución Mean NI and S/P (%) ratio in calves immunized with SPV vaccines stabilized with different concentrations of Carbopol®.

WPV	Group 1		Group 2		Group 3		Group 4		Group 5	
	NI	S/P(%)	NI	S/P(%)	NI	S/P(%)	NI	S/P(%)	NI	S/P(%)
0	0.50	5	0.50	5	0.50	5	0.50	5	0.50	5
1WPV	1.00	15	1.00	17	1.25	19	1.00	17	0.25	5
2WPV	1.75	32	1.75	35	2.00	35	1.75	35	0.25	5
3WPV	2.25	*41	2.75	*45	2.50	*47	2.25	*41	0.25	5
4WPV	*2.50	38	*3.00	42	*3.0	41	*2.50	38	0.25	5
5WPV	2.50	37	2.75	45	2.75	45	2.50	39	0.25	5
6WPV	2.50	35	2.75	44	3.0	45	2.50	36	0.25	5

WPV: weeks post vaccination. NI = Neutralization index (NI ≥ 1.5 protective). S/P = sample to positive ratio (S/P >30 protective). *: peak of the antibody immune response. Group (1): calves vaccinated with 0.25% Carbopol® stabilized SPV vaccine. Group (2): calves vaccinated with 0.5% Carbopol® stabilized SPV vaccine. Group (3): calves vaccinated with 1% Carbopol® stabilized SPV vaccine. Group (4): calves vaccinated with lactalbumin hydrolysate/sucrose stabilized SPV vaccine. Group (5): control non-vaccinated calves.



Fig. 1. Final freeze-dried vials for the SPV vaccine stabilized with 0.5% Carbopol® are capped with orange flip-off caps on the right side; whereas, the SPV vaccine stabilized with lactalbumin hydrolysate/sucrose are green capped on the left side.

while reduction in titer was higher ($0.5 \log_{10} \text{TCID}_{50}/\text{mL}$) when lactalbumin/hydrolysate sucrose stabilizer was used (Table 2).

Thermo stability tests of the prepared vaccines

Thermo stability of the prepared lyophilized SPV vaccine with 0.5% Carbopol® (concentration of choice for use in SPV vaccine) and lactalbumin hydrolysate/sucrose stabilizer kept at 37°C for one week, showed reduction in virus titer as $2 \log_{10} \text{TCID}_{50}/\text{mL}$ and $2.5 \log_{10} \text{TCID}_{50}/\text{mL}$, respectively, as shown in Table 3.

Sterility test

All prepared SPV vaccine stabilized with Carbopol® and lactalbumin hydrolysate/sucrose were proved to be free from any bacterial, fungal and mycoplasma contamination.

Safety test

Inoculation of 10X Carbopol® stabilized SPV vaccine in calves proved the prepared vaccine was safe to be used in calves, since the vaccinated calves did not show systemic or local reactions.

Cell mediated immune response of calves vaccinated with Carbopol® and lactalbumin hydrolysate/sucrose stabilized SPV vaccines

The highest level of cell proliferation was determined at day 10 in all vaccinated groups. Increasing mean OD values (1.855 and 1.858) were observed in groups 2 and 3 vaccinated with 0.5 and 1% Carbopol® stabilized SPV vaccines, respectively. Mean OD 1.500 was obtained for calves vaccinated with lactalbumin hydrolysate/sucrose stabilized SPV vaccine (group 4), while low OD (that not exceed 0.087) was read in control non-vaccinated (group 5), as shown in Table 4.

Determination of the optimal dose of prepared SPV vaccine

The results of mean NI and S/P ratio obtained for different doses of SPV vaccines are shown in Table 5. Protective levels of antibodies (NI=3 and S/P ratio (47% and 48%) were induced in calves vaccinated with $10^{3.5} \text{TCID}_{50}$ and 10^4TCID_{50} 0.5% carbomer stabilized SPV vaccines (groups 6, 7), respectively; while in calves vaccinated with the $10^{3.5} \text{TCID}_{50}$ and 10^4TCID_{50} lactalbumin hydrolysate/sucrose stabilized SPV vaccines (groups 8 and 9) the detected NI value was 2.5, in both groups

Table 2. SPV vaccines titers before and after lyophilization.

SPV stabilized vaccine	Virus titer $\log_{10} \text{TCID}_{50}/\text{mL}$	
	Before lyophilization	After lyophilization
SPV vaccine without stabilizer	5.5	-
SPV vaccine with 0.25 Carbopol®	5.5	5.25
SPV vaccine with 0.5% Carbopol®	5.5	5.25
SPV vaccine with 1% Carbopol®	5.5	5.25
SPV vaccine with 5% lactalbumin hydrolysate and 2.5% sucrose	5.5	5.0

Table 3. Thermo stability of the prepared SPV vaccines.

Time of titration	Virus titer ($\log_{10}$ TCID ₅₀ /mL)			
	SPV 10 ^{5.5} TCID ₅₀ /mL		Reduction in virus titer	
	0.5% Carbopol®	Lactalbumin hydrolysate/sucrose	0.5% Carbopol®	Lactalbumin hydrolysate/sucrose
Before lyophilization	5.5	5.5	0.0	0.0
After lyophilization	5.25	5.0	0.25	0.5
1 st DPL *	5.25	5.0	0.25	0.5
2 nd DPL	5.0	4.75	0.5	0.75
3 rd DPL	5.0	4.75	0.5	0.75
4 th DPL	4.5	4.25	1.0	1.25
5 th DPL	4.5	4.0	1.0	1.5
6 th DPL	4.25	3.75	1.25	1.75
7 th DPL	3.5	3.00	2.0	2.5

*DPL: days post lyophilization.

Table 4. Optical density (OD) of cell mediated immune response in calves vaccinated with 0,5% and 1% Carbopol® and lactalbumin hydrolysate/sucrose SPV vaccines.

Calves' groups	Calf number	OD of cell mediated immune response on DPV*								
		0	1DPV	3DPV	5DPV	7DPV	*10DPV	14DPV	21DPV	28DPV
Group 2	1	0.082	0.281	0.451	0.671	0.825	**1.853	1.654	0.831	0.413
	2	0.089	0.271	0.471	0.692	0.862	**1.862	1.684	0.751	0.407
	3	0.092	0.321	0.467	0.683	0.913	**1.852	1.623	0.837	0.475
	Average	0.087	0.291	0.463	0.682	0.866	**1.855	1.653	0.806	0.431
Group 3	1	0.079	0.287	0.462	0.685	0.883	**1.838	1.662	0.853	0.438
	2	0.087	0.261	0.460	0.643	0.860	**1.819	1.609	0.743	0.401
	3	0.083	0.323	0.485	0.717	0.893	**1.917	1.783	0.846	0.447
	Average	0.083	0.290	0.469	0.681	0.878	**1.858	1.684	0.814	0.428
Group 4	1	0.075	0.162	0.264	0.381	0.650	**1.490	0.953	0.751	0.362
	2	0.065	0.093	0.247	0.395	0.618	**1.415	1.025	0.724	0.382
	3	0.080	0.164	0.289	0.401	0.738	**1.596	1.184	0.764	0.319
	Average	0.073	0.139	0.266	0.373	0.668	**1.500	1.054	0.746	0.354
Group 5	1	0.084	0.081	0.087	0.085	0.082	0.083	0.086	0.084	0.087
	2	0.081	0.079	0.085	0.083	0.081	0.085	0.087	0.081	0.083
	3	0.073	0.078	0.74	0.081	0.076	0.084	0.079	0.085	0.080
	Average	0.079	0.079	0.082	0.083	0.079	0.084	0.081	0.083	0.083

DPV*: days post vaccination. **: highest OD. Group 2: calves vaccinated with SPV vaccine stabilized with 0.5% Carbopol®. Group 3: calves vaccinated with SPV vaccine stabilized with 1.0% Carbopol®. Group 4: calves vaccinated with lactalbumin hydrolysate/sucrose SPV vaccine. .

Table 5. Mean NI and S/P ratio of vaccinated calves with different doses of SPV vaccine.

WPV	Grupo 6		Grupo 7		Grupo 8		Grupo 9		Grupo 5	
	NI	S/P(%)	NI	S/P(%)	NI	S/P(%)	NI	S/P(%)	NI	S/P(%)
0	0.50	5	0.50	5	0.50	5	0.50	5	0.50	5
1	1.00	16	1.25	18	1.00	17	1.25	20	0.25	5
2	2.00	33	1.75	34	1.75	35	1.75	38	0.25	5
3	2.50	*47	2.75	*48	2.25	*40	2.25	*42	0.25	5
4	*3.00	42	*3.0	41	*2.50	37	*2.50	38	0.25	5
5	2.75	46	2.75	47	2.25	38	2.50	36	0.25	5
6	2.75	44	3.0	45	2.50	36	2.50	37	0.25	5

WPV: weeks post vaccination. NI: neutralization index ($NI \geq 1.5$ protective). S/P: sample to positive ratio ($S/P > 30\%$ protective). *: peak antibody levels. Group 6: vaccinated with a dose of $10^{3.5}$ TCID₅₀/animal (Carbopol® stabilized SPV vaccine). Group 7: vaccinated with a dose of 10^4 TCID₅₀/animal (Carbopol® stabilized SPV vaccine). Group 8: vaccinated with a dose of $10^{3.5}$ TCID₅₀/animal (lactalbumin stabilized SPV vaccine). Group 9: vaccinated with a dose of 10^4 TCID₅₀/animal (lactalbumin stabilized SPV vaccine). Group 5: non-vaccinated group.

and the S/P ratio was 40% and 42%, respectively. The optimal dose of choice of the SPV vaccine was $10^{3.5}$ TCID₅₀/animal.

Discussion

LSD is a disease of economic importance that affects cattle. The use of heterologous vaccination with SPV vaccine is a LSDV infection control strategy. An incomplete protection with SPV vaccine is obtained,⁽⁶⁾ then the use of Carbopol® as adjuvant stabilizer can improve such heterologous vaccination.

The highest level of antibodies detected by NI and S/P in calves vaccinated with different concentrations of Carbopol® were obtained in groups 2 and 3 that received 0.5% and 1% Carbopol® stabilized SPV vaccine. Therefore, 0.5% carbomer was the concentration of choice to be used with SPV vaccine ($10^{3.5}$ TCID₅₀/field dose). Similar results were obtained for different animal viral vaccines like lyophilized live-attenuated NDV vaccine LaSota, freeze-dried inactivated bovine viral respiratory combined vaccine, modified-life vaccine Ingelvac PRRS1 MLV, tissue culture inactivated rabies vaccine, inactivated equine herpes virus-1 and freeze-dried bovine ephemeral fever virus vaccine.^(9,10,11,12,13)

The vaccine stabilizer is a corner stone in maintaining the vaccine efficacy specially when exposed to high temperature; it plays an important role in prolongation of the shelf life of vaccine. The lyophilized Carbopol® SPV vaccine showed several advantages than the usual prepared lactalbumin hydrolysate/sucrose SPV vaccine, since it exhibited better physical properties and formation of whitish uniform compact solid disks

on contrast to the yellowish, vacuolated contracted disk in case of using lactalbumin hydrolysate/sucrose stabilizer. It can be explained on the basis that the Carbopol® polymer is able to retain and absorb water to form agglomerates of polymer chains that are irreversible,^(8,17) and makes a high uniformity white compact disk of prepared NDV vaccine when carbomer is used in 0.5% concentration, in comparison to the skimmed milk stabilizer.⁽¹⁰⁾

In addition to the physical properties, the prepared Carbopol® stabilized vaccine maintained a higher virus titer after lyophilization ($10^{5.25}$ TCID₅₀/mL) compared to lyophilized lactalbumin hydrolysate/sucrose SPV vaccine ($10^{5.0}$ TCID₅₀/mL); in addition, minor virus reduction was obtained when exposed at 37°C for one week in thermo stability test, since the decrease in virus titer for Carbopol® stabilized SPV vaccine was 2 log₁₀ TCID₅₀/mL, while in case of SPV vaccine prepared by using lactalbumin hydrolysate/sucrose stabilizer, was 2.5 log₁₀ TCID₅₀/mL. Similar results were obtained by the prepared freeze-dried bovine viral respiratory combined vaccine using 0.5% carbomer as stabilizer. Carbomer binds to virus particles and forms a tight sealed that protects the integrity of the formed complex, even after heat exposure, keeping the thermo stability properties of the prepared vaccine.⁽¹³⁾

The testing of the prepared SPV vaccine stabilized with Carbopol® indicated its freedom of fungal and bacterial contamination. Furthermore, none of the vaccinated calves with the 10x field dose experienced any systemic or local reactions or mortality; this agreed with the information provided in the Carbopol® safety data sheet and indicates the non-toxic properties of carbomer when used as a stabilizer.⁽⁷⁾

The immune response to capripox vaccination and infection is mainly cell mediated. One the most important components in capripox cellular immune response in dermal layer are dendritic cells, which are characterized by induction of IFN- γ .⁽¹⁸⁾ Due to biosafety requirements, the cellular immune response for the prepared SPV vaccine was measured, instead of challenge test. The highest values of cell proliferation were detected at 10th day PV in all vaccinated groups, with an increase in mean OD values (1.855 and 1.858) in calves vaccinated with 0.5 and 1% Carbopol[®] stabilized SPV vaccines (group 6 and 7), respectively, compared to group 8 (vaccinated with the lactalbumin hydrolysate/sucrose SPV vaccine) that showed a mean OD 1.500, showing the great effect of Carbopol[®] on cellular immunity. This carbomer stimulates CD8 T-cell through induction of a different metabolic state in dendritic cells that produce more IL-1 β and IL-18; also Carbopol[®] induces early IFN γ which stimulates T-cell differentiation into specific effector phenotypes.^(7, 9,14)

Serum samples collected from calves vaccinated with different field doses of the SPV vaccine (10^{3.5} and 10⁴ TCID₅₀/field dose) stabilized with Carbopol[®] (groups 6 and 7) and lactalbumin/hydrolyzate sucrose (groups 8 and 9), were evaluated based on serological tests. The results of the mean NI and S / P ratios showed protective antibody levels in all vaccinated calves. Regarding to the mean NI and S/P obtained for 10^{3.5} and 10⁴ TCID₅₀/field doses, the dose of choice for the SPV vaccine was 10^{3.5} TCID₅₀/field dose; this result corresponds with the recommendation that a 10X field dose of SPV vaccine used for sheep against SPV, develops sufficient protection against LSD in cattle.^(4,5,19)

Conclusion

The use of Carbopol[®] not only enhanced the physical properties of the prepared lyophilized SPV vaccines, but it also increased and improved the humoral and cellular immune response of vaccinated calves and could overcome the incomplete protection of heterologous vaccination with SPV vaccine against LSD.

Conflict of interest

The authors whose names are listed above certify that they have no affiliations with or involvement in any organization or entity with any financial and nonfinancial interest in the subject matter or materials discussed in this manuscript.

Contributors

Ayatollah. I. Ibrahim: study design, sampling, methodology, interpretation of results and writing the manuscript.

Doaa I. Rady: study design, sampling, methodology, interpretation of results and writing the manuscript.

Eman R. Abdo: study design, sampling, methodology, interpretation of results and writing the manuscript.

Alaa A. El-Kholy: study design, sampling, methodology, interpretation of results and writing the manuscript.

All authors approved the final version of the manuscript.

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Eficacia comparativa de diferentes concentraciones de Carbopol como estabilizador de la vacuna viva atenuada contra el virus de la viruela ovina contra la dermatosis nodular contagiosa

Resumen

En Egipto, la vacuna atenuada liofilizada contra el virus de la viruela ovina ha sido utilizado para la vacunación del ganado, contra el virus de la dermatosis nodular contagiosa, para controlar su impacto económico en la industria ganadera. En este trabajo, validamos la eficacia del Carbopol®, como estabilizador y adyuvante, para mejorar la inmunogenicidad de dicha vacuna heteróloga contra la dermatosis nodular contagiosa. La liofilización de la vacuna contra el virus de la viruela ovina estabilizada con Carbopol®, resultó en mejores propiedades físicas y antigénicas que la liofilización con el estabilizador de lactoalbúmina/sacarosa; lo anterior se manifestó en la uniformidad superior del disco, la termoestabilidad a 37°C y la menor reducción del título del virus. La inmunización de grupos de terneros con dosis variables de vacuna contra el virus de la viruela ovina, que contenían diferentes concentraciones de Carbopol®, reveló que la dosis de campo de elección fue 10^{3.5} TCID₅₀ de la vacuna contra el virus de la viruela ovina conteniendo 0,5% de Carbopol®, la que indujo un índice de neutralización sérica protectora de 2,5 y una relación S/P de ELISA de 36 a la cuarta semana después de la vacunación. Además, la inclusión de Carbopol® al 0,5% en la formulación de la vacuna contra el virus de la viruela ovina fue segura en los bovinos y potenció la respuesta inmunitaria celular contra el virus de la dermatosis nodular contagiosa, como lo demuestra el aumento de la proliferación de células T. Por lo tanto, se recomienda el uso de Carbopol® al 0,5% en la preparación de la vacuna viva atenuada contra el virus de la viruela ovina para conferir una mejor protección contra la infección por el virus de la dermatosis nodular contagiosa.

Keywords: dermatosis nodular contagiosa; virus de la viruela de la oveja; vacunación; inmunidad celular; ELISA.