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Use of Carbopol as an adjuvant in preparation of inactivated rabbit pasteurellosis vaccine

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ABSTRACT

The current study was designed to investigate the immunological response of rabbits vaccinated with the rabbit pasteurellosis vaccine adjuvanted with Carbopol®. One hundred and twenty, 2 weeks old rabbits were divided into four groups, 30 for each. The first group was received the formalized *P. multocida* vaccine. The second group was received the Montanide ISA206 *P. multocida* vaccine. The third group was received the Carbopol *P. multocida* vaccine. The fourth group was left unvaccinated as a control group. The prepared vaccines were evaluated by the measurement of the antibody response by the indirect haemagglutination (IHA) and ELISA techniques. The vaccines' potency was assessed using the challenge test against the virulent strains of *P. multocida* serotypes A and D. The findings revealed that the Carbopol® rabbit pasteurellosis vaccination elicited a strong and long-lasting antibody response, as well as considerable protection against the virulent strains of *P. multocida* serotypes A and D.

1. INTRODUCTION

Pasteurellosis, caused by *Pasteurella multocida*, is one of the most serious bacterial illnesses of rabbits, causing major economic losses in big production facilities across the world (Takashima et al., 2001). The upper respiratory tract is the most common location of first infection. Transfer is easily accomplished by direct contact between vulnerable rabbits and carrier animals, as well as aerial transfer. Crowding, traffic, and high ammonia concentrations in the air are common stressors that induce latent *P. multocida* to multiply and produce illness (Di Giacomo et al., 1991). The illness is characterized by a variety of clinical signs, including respiratory distress, vaginal infections, abscesses, otitis, and septicemia, however *P. multocida* infection can occur without pasteurellosis (Dabo et al., 1999). Pasteurellosis is prevented in Egypt by vaccination with whole-cell bacterin, which provides serotype-specific protection, or with live vaccines made up of attenuated strains, which protect against both homologous and heterologous serotypes (Wang and Glisson, 1994). The development of effective vaccines will necessitate a combination of approaches, including the identification of appropriate adjuvants that will present the antigen in a way that allows for the induction of a sufficient and competent immune response with minimal to no adverse effects on recipients (Gartlan et al., 2016). Furthermore, the adjuvant must be pharmaceutically stable, cost-effective, and trustworthy, with a low cost per dosage and a low risk-to-safety ratio (Kaurav*et al.* 2018). Carbopol® has been studied as an adjuvant in veterinary vaccinations, which are not hazardous to animals and are more effective than antigen alone (Mumford et al., 1994). Carbopol® boosts cellular immunity by promoting T helper (Th1) polarization and interferon-gamma (IFNγ) production, as well as antigen uptake by macrophages (Gartlan et al., 2016). Carbopol® adjuvant increased the intensity and durability of antibody responses induced by an inactivated vaccination (Zhang et al., 2018a). The purpose of this study was to prepare and evaluate an inactivated rabbit pasteurellosis vaccine adjuvanted with Carbopol®, as well as to compare the effectiveness of the Carbopol® vaccine to that of a formalized inactivated vaccine and Montanide ISA206 vaccine.

2. MATERIAL AND METHODS

2.1. Ethical approval

The Research Committee of the Veterinary Serum and Vaccine Research Institute, Abasia, Agricultural Research Centre (VSVRI/ARC), Cairo, Egypt, approved the current study

2.2. P. multocida strains

P. multocida serotypes A and D were used in the preparation of different rabbit pasteurellosis vaccines. The strains were supplied by Aerobic Bacterial Vaccines Department, Abbasia, Cairo

2.3. Experimental animals (rabbits and mice)

A total of 8 rabbits about 2 weeks of age (1-1.5) kg body weight were obtained from the Animal House Farm of Veterinary Serum and Vaccine Research Institute (VSVRI) were used for the passage of *P. multocida* strains, and 120 rabbits were used for evaluation of the prepared vaccines. Rabbits were not previously vaccinated or received antibiotics and reared according to biosafety and biosecurity rules

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A total of 20 Swiss white mice (25-30) gm body weight were used for evaluation and safety of the prepared vaccines

2.4. Adjuvants

Montanide ISA206 (Seppic, France) is a mineral oil based adjuvant from complex water in oil emulsion; it was used by the ratio 50/50.

Carbopol®, Lubrizol supplied the powder, which was mixed in hot water to create a 1% aqueous stock solution (United States Pharmacopeial Convention Inc, 2022). The produced solution was autoclaved at 121°C for 20 minutes and kept at 4°C for future use

2.5. Vaccines preparation

2.5.1. Preparation of formalized inactivated P. multocida vaccine

Serotypes of *P. multocida* A and D were cultured separately in tryptic soya broth (TSB) at 37˚C for 24 hrs with shaking. The culture was standardized to 4×10^9 C FU/ml for each strain according to (Mukkur et al., 1982). The bacteria were inactivated with 0.5% formalin (Fisher Scientific, UK, Belgium) in a concentration of 37% and incubated at 37˚C for 24 hrs. The prepared bacterin was tested for purity, safety and sterility as mentioned by (OIE, 2012). Finally, culture mixed with 0.01% thiomersal and stored at 4˚C until used

2.5.2. Preparation of Montanide ISA 206 P. multocida vaccine

The bacterin of *P. multocida* was mixed in equal volume with Montanide ISA206 in a ratio of 50/50 (W/O). Finally, the thiomersal was added at a final concentration of 0.01% and stored at 4˚C until used (Mukkur et al., 1982)

2.5.3. Preparation of Carbopol® P. multocida vaccine

The bacterin of *P. multocida* was mixed in equal volume with Carbopol® in a ratio of 50/50. Finally, the thiomersal was added at a final concentration of 0.01% and stored at 4˚C until used (United States Pharmacopeia, 2022)

2.6. Quality control of the prepared vaccines

The prepared vaccines were assessed for purity, sterility and safety according to OIE, 2012

2.7. Experimental design

A total of 120 rabbits, two-week old were reared under complete hygienic measures and were showing no history of previous infection or vaccination. The experimental rabbits were divided into four groups; 30 for each. The first group was received the formalized *P. multocida* vaccine. The second group was received the Montanide ISA206 *P. multocida* vaccine, whereas the third received Carbopol® *P. multocida* vaccine. The fourth group was left unvaccinated (control group). The rabbits were inoculated with 2 ml of vaccine, S/C, given in two doses separated by one month. All rabbit groups were challenged with virulent strains of *P. multocida* 21 days following booster vaccinations. Serum samples were collected for determination of the humoral immune response of the vaccinated rabbits by IHA according to OIE Terrestrial Manual, 2008 and by ELISA according to Barrow, 1992. The potency of the prepared vaccines was evaluated by the challenge test against the virulent strains of *P. multocida* serotypes A and D according to OIE, 2012

2.8. Assessment of the humoral immune response

2.8.1. Indirect Hemagglutination Test (IHA)

It was carried according to OIE Terrestrial Manual, 2008 for measuring antibody titers against *P. multocida*types A and D in vaccinated rabbits using Glutaraldehyde- fixed sheep erythrocytes (GA-SRBC) and capsular antigens of *P. multocida* types A and D

2.8.2. ELISA test

The test was performed for determination of the antibody titers by using serum samples of the vaccinated rabbits according to Barrow, 1992

2.9. Challenge test

The test was used to evaluate the protection % (P %) of the vaccinated rabbits against the challenge with the virulent strains of *P. multocida* serotypes A and D. according to OIE, 2012

3. RESULTS

3.1. Quality control of the prepared vaccines
Sterility tests confirmed that the prepared vaccines were devoid of bacterial, fungal, and mycoplasma contamination. The prepared vaccines were proved to be safe; there were no local or systemic post-injection reactions for 15 days of observation

3.2. Indirect Hemagglutination Test (IHA)
IHA investigated the humoral immune response of rabbits inoculated with various *P. multocida* vaccines as illustrated in Tables 1 and 2 noticed that a significant increase of the overall means of the antibody titers against *P. multocida* by IHA test was in group of rabbits vaccinated with Carbopol® vaccine

Table (1) Antibody titers against *P. multocida* type "A" in rabbits vaccinated with different types of adjuvants by IHA

Interval periods for serum collection	Types of vaccines			
	G1	G2	G3	G4
Pre-vaccination	2	2	2	2
1st vaccination				
Two weeks after 1st vaccination	64	128	128	2
Booster vaccination				
Two weeks after 2nd vaccination	128	256	256	2
Challenge				
Two weeks after the challenge	128	128	128	2
Four weeks after the challenge	128	512	512	2
Six weeks after the challenge	512	1024	1024	2
Eight weeks after challenge	256	512	512	2
Ten weeks after the challenge	128	256	512	2
Twelve weeks after the challenge	128	256	256	2
Fourteen weeks after the challenge	64	128	128	2
Overall means	154	320	346	2

G1: Formalized *P. multocida* vaccine, G2: *P. multocida* vaccine adjuvanted with Montanide ISA206, G3: *P. multocida* vaccine adjuvanted with Carbopol® ,G4: Control (non-vaccinated), 1st vaccination: at four weeks of age, Booster vaccination: at eight weeks of age, Challenge: at eleven weeks of age

Table (2) Antibody titers against *P. multocida* type "D" in rabbits vaccinated with different types of adjuvants by IHA

Interval periods for serum collection	Types of vaccines			
	G1	G2	G3	G4
Pre-vaccination	2	2	2	2
1st vaccination				
Two weeks after 1st vaccination	32	64	64	2
Booster vaccination				
Two weeks after 2nd vaccination	64	256	256	2
Challenge				
Two weeks after the challenge	64	128	128	2
Four weeks after the challenge	128	512	512	2
Six weeks after the challenge	256	512	512	2
Eight weeks after the challenge	128	256	256	2
Ten weeks after the challenge	64	128	128	2
Twelve weeks after the challenge	32	128	128	2
Fourteen weeks after the challenge	32	32	32	2
Overall means	80	202	202	2

G1: Formalized *P. multocida* vaccine, G2: *P. multocida* vaccine adjuvanted with Montanide ISA206, G3: *P. multocida* vaccine adjuvanted with Carbopol® ,G4: Control (non-vaccinated), 1st vaccination: at four weeks of age, Booster vaccination: at eight weeks of age, Challenge: at eleven weeks of age

3.3. ELISA test

The humoral immune response of rabbits inoculated with several *P. multocida* vaccines was investigated by ELISA as illustrated in Tables 3 and 4 noticed that a significant increase of the overall means of the antibody titers against *P. multocida* by ELISA test was in group of rabbits vaccinated with Carbopol® vaccine

3.4. Challenge test

The produced vaccines' potency was tested by the challenge test against *P. multocida* in rabbits inoculated with several *P. multocida* vaccines were illustrated in Tables 5 and 6 showed that the protection % against the challenge with *P. multocia* was 100% for rabbit pasteurellosis vaccine adjuvanted with Carbopol®

Table (3) Antibody titers against *P. multocida* type "A" in rabbits vaccinated with different types of adjuvants by ELISA

Interval periods for serum collection	Types of vaccines			
	G1	G2	G3	G4
Pre-vaccination	20	20	20	20
1st vaccination				
Two weeks after 1st vaccination	157	241	360	20
Booster vaccination				
Two weeks after 2nd vaccination	729	965	996	20
Challenge				
Two weeks after the challenge	1039	1636	1902	20
Four weeks after the challenge	2423	3665	4166	20
Six weeks after the challenge	2541	3927	4958	20
Eight weeks after challenge	2106	3229	3551	20
Ten weeks after the challenge	1624	2199	2768	20
Twelve weeks after the challenge	1010	1487	1860	20
Fourteen weeks after the challenge	743	892	969	20
Overall means	1239	1826	2155	20

G1: Formalized *P. multocida* vaccine, G2: *P. multocida* vaccine adjuvanted with Montanide ISA206, G3: *P. multocida* vaccine adjuvanted with Carbopol® ,G4: Control (non-vaccinated), 1st vaccination: at four weeks of age, Booster vaccination: at eight weeks of age, Challenge: at eleven weeks of age

Table (4) Antibody titers against *P. multocida* type "D" in rabbits vaccinated with different types of adjuvants by ELISA

Interval periods for serum collection	Types of vaccines			
	G1	G2	G3	G4
Pre-vaccination	10	10	10	10
1st vaccination				
Two weeks after 1st vaccination	157	241	360	10
Booster vaccination				
Two weeks after 2nd vaccination	729	965	996	10
Challenge				
Two weeks after the challenge	1039	1636	1902	10
Four weeks after the challenge	2423	3665	4166	10
Six weeks after the challenge	2541	3927	4958	10
Eight weeks after challenge	2106	3229	3551	10
Ten weeks after the challenge	1624	2199	2768	10
Twelve weeks after the challenge	1010	1487	1860	10
Fourteen weeks after the challenge	743	892	969	10
Overall means	1238	1825	2154	10

G1: Formalized *P. multocida* vaccine, G2: *P. multocida* vaccine adjuvanted with Montanide ISA206, G3: *P. multocida* vaccine adjuvanted with Carbopol® ,G4: Control (non-vaccinated), 1st vaccination: at four weeks of age, Booster vaccination: at eight weeks of age, Challenge: at eleven weeks of age

Table (5) Challenge test against P. multocida type ‘‘A’’ in rabbits vaccinated with different types of adjuvants

Types of vaccines	G1	G2	G3	G4
Total no. of rabbits	15	15	15	15
D	1	0	0	15
S	14	15	15	0
P %	93	100	100	0

P% =No. of survived rabbits/Total No. of rabbitsX 100
S= Survived rabbits, **D=**Dead rabbits, **G1**: Formalized *P. multocida* vaccine, **G2***: P. multocida* vaccine adjuvanted with Montanide ISA206,**G3:** *P. multocida* vaccine adjuvanted with Carbopol®, **G4:** Control (non-vaccinated)

Table (6) Challenge test against P. multocida type ‘‘D’’ in rabbits vaccinated with different types of adjuvants

Types of vaccines	G1	G2	G3	G4
Total no. of rabbits	15	15	15	15
D	0	0	0	15
S	15	15	15	0
P %	100	100	100	0

P% =No. of survived rabbits/Total No. of rabbitsX 100
S= Survived rabbits, D=Dead rabbits, G1: Formalized *P. multocida* vaccine, G2*: P. multocida* vaccine adjuvanted with Montanide ISA206,G3: *P. multocida* vaccine adjuvanted with Carbopol®, G4: Control (non-vaccinated)

4. DISCUSSION

The rabbit business, as one of the tiny livestock, has a particular economic potential to help solve the meat crisis after the poultry sector (Mohammed et al., 2013). Several bacteria from the Pasteurellaceae family are potential rabbit infections. *P. multocida* is particularly important, and outbreaks induced by this species produce significant economic losses in rabbits (Anina et al., 2009). Carbopol® is a synthetic polymer with several applications in medicines. The aqueous Carbopol® gel is thermostable, suitable with a wide range of substances, and flows freely through a variety of application channels (Islam et al., 2004). Carbopol® offers several benefits, including excellent safety, nontoxicity, and suspending properties (Ahuja et al., 1997). The advantages of employing aquatic Carbopol® gel include its simple flow, affinity for numerous active substances, and heat stability (Zhang et al., 2018b). So this study was done to develop an inactivated rabbit pasteurellosis vaccine adjuvanted with Carbopol® and compare its potency to established formalized inactivated vaccine and Montanides ISA206 vaccine. The humoral

immune response of rabbits vaccinated with various *P. multocida* vaccines was evaluated using IHA, as shown in Tables 1 and 2. It was discovered that there was a significant increase in the overall means of antibody titers against *P. multocida* by IHA test in the group of rabbits vaccinated with Carbopol®. Zhang et al., 2018a found that the *P. multocida* vaccination against progressive atrophic rhinitis (PAR) adjuvated with Carbopol® 971 elicited high titers of serum neutralization test (SNT) (1:64) and high levels of tumor necrosing factor (TNF-α), interleukin (IL-6), and IL-17A in mice injected with the vaccine. Furthermore, Naglaa et al., 2023 observed that the freeze-dried combination vaccination against Rift Valley fever and bovine ephemeral fever including Carbopol® elicited a strong humoral immune response. Furthermore, Gartlan et al., 2016 reported that Carbopol® can enhance and activate cellular and humoral immune responses in animals. The humoral immune response of the vaccinated rabbits with different *P. multocida* vaccines was evaluated by ELISA as illustrated in Tables 3 and 4 noticed that a significant increase of the overall means of the antibody titers against *P. multocida* by ELISA test was in group of rabbits vaccinated with Carbopol® vaccine. These data agreed with Zhang et al., 2018a who reported that PAR vaccine adjuvated with Carbopol 971 elicited both protective humoral and cellular immune response against PAR. Moreover, Maha et al., 2019 recorded that the combination inactivated pneumo-4 vaccine including bovine viral diarrhoea, infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI-3), and bovine respiratory syncytial virus (BRSV) adjuvanted with Carbopol®induced high and long duration of antibody response and elicited high cellular immune response. AlsoAbd El-Moneam et al., 2020 stated that the live-attenuated LaSota vaccine adjuvanted with Carbopol® 940 induced high cellular and humoral immune response. The potency of the vaccines was evaluated by the challenge test against *P. multocida* in rabbits vaccinated with different *P. multocida* vaccines was illustrated in Tables 5 and 6 showed that the P% against the challenge with *P. multocida* was 100% for rabbit pasteurellosis vaccine adjuvanted with Carbopol®. These findings were consistent with those of Zhang et al., 2018a who concluded that the PAR vaccine adjuvated with carbopol 971 provides good protection against PAR and *P. multocida* infections, and mice immunised with Carbopol® vaccine had no detectable pathological changes in snouts or organs after challenge. Also these data were partially agreed with Maha et al., 2019 who reported that pneumo-4 vaccine adjuvanted with Carbopol® 0.5% was pure and completely safe to be used in calves and be considered highly potent along 6 months after second booster dose. Moreover, Abd El-Moneam et al., 2020 concluded that the live-attenuated LaSota vaccine adjuvanted with Carbopol® 940 induced 100% protection against challenge with virulent NDV post 21 days after vaccination and the antibody titer was prolonged until 6 weeks post vaccination.

5. CONCLUSIONS

From the obtained results it could be concluded that the rabbit pasteurellosis vaccine adjuvanted with Carbopol® induced a considerable immunity in rabbits as it gave high and long duration of antibody response. Also, it was efficient and safe in protection of rabbits against *P. multocida* infection. Depending on this study, it could be suggested to use this Carbopol® vaccine for control of *P. multocida* infection in rabbit's industry.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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