Benha Veterinary Medical Journal 45 (2023) 168-172

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Evaluation of different adjuvants to the inactivated PPR vaccine

Manal Abd El-Aziz[1,2*], Gabr F. El-Bagoury[1], Mohammed H. Khodeir[2]

[1] *Department of Virology, Faculty of Veterinary Medicine, Benha University, Egypt*

[2] *Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt*

ARTICLE INFO

Keywords

PPRV

Inactivated vaccine

Montanide, Carbomer

Received 09/11/2023

Accepted 25/11/2023

Available On-Line 31/12/2023

ABSTRACT

The Peste des petits ruminant (PPR) is a viral devastating illness of sheep and goats resulting in huge losses to the economy and its control mainly depends on successful vaccination of susceptible hosts. The use of inactivated vaccine is preferable in non-endemic regions. Determination of the best adjuvant to the inactivated PPR vaccine induces the highest vaccine potency. Preparation of inactivated PPR virus infected fluid obtained from infected Vero cell by binary ethylene amine and divided it into three portions adjuvant by Montanide -01 gel; Montanide oil ISA 206 and Carbomer then subjected to quality control testing. All of the three prepared inactivated PPR vaccine formulae were found to be free from foreign contaminants; safe and highly immunogenic inducing nearly similar antibody titers in vaccinated sheep remaining up to 28 weeks post vaccination and suggested to be of longer duration. Each of Mantonide-01 gel; Montanide oil ISA 206 and Carbomer could be recommended to use as an adjuvant to the inactivated PPR vaccine with regard to the cost of vaccine production.

1. INTRODUCTION

A viral disease known as pest des petits ruminants (PPR) has a major negative economic impact on small ruminant livelihoods. Thus, throughout Asia, Africa, and the Middle East, it is regarded as one of the most devastating animal illnesses (OIE and FAO, 2015). In Egypt, the first PPR outbreak was in 1987 in Giza governorate (Ikram et al., 1988). PPRV is still circulating in Egypt, where it causes epidemics in small ruminants, who are its major host (Maged et al., 2018), and reappeared in some Egyptian governorates (Ahmed et al., 2021). Animal vaccination mainly aims to create an immune response that provides long-term protection against infection by imitating the natural immunity of non-pathogenic constituents. (Meeusen et al., 2007). Immunization is essential aim to keep animals safe and protect them from infectious diseases. For the purpose of eliminating fatal illnesses and enhancing the effectiveness of vaccinations in terms of inconsistent or partial protection, safe and optimal vaccine development is necessary. With the addition of new adjuvants, a vaccine's ability to provide greater immunity can be increased for fighting pathogens of high antigenic diversity (Verma et al., 2023). Since inactivated vaccines do not carry the risk of reversion to virulence or disease transmission associated with live attenuated vaccinations, they are frequently chosen in non-endemic areas. In order to properly inactivate different viruses for the creation of vaccines, binary ethylene amin (BEI) reacts with viral nucleic acids while preserving epitope accessibility and conformation (Banhnemann, 1990 and Razmaraii et al., 2012). The inactivated PPR vaccine is commonly prepared by BEI containing the viral antigen (Ronchi et al., 2016). The use of adjuvants can strengthen the body's immune responses and alter the nature of immune responses when combined with antigen(s), as well as promote non-specific immunity (Foumani et al., 2012 and Jin et al., 2019). Adjuvants used in veterinary vaccines are absolutely necessary. A modest antibody response is induced by immunizing animals with pure protein antigens; several immunizations may be necessary to produce a significant antibody response. In vaccine candidates, adjuvants increase the efficacy of weak antigens, boosting immune responses that would not be sufficiently elicited in their absence and allowing for the use of lower vaccine doses (Reed et al., 2013). The optimum adjuvant, according to Ibrahim (2011) and Daoud et al. (2013), should prompt the cellular immune response in addition to stimulating the humoral immune response early and inducing high antibody titers over time. In continuous aqueous phase emulsions known as Water-in-Oil-in-Water (W/O/W) formulas, oil droplets contain a secondary aqueous phase, or double emulsion. SEPPIC W/O/W adjuvants are compatible with inactivated and recombinant antigens. Vaccines adjuvanted with Montanide ISA 206 oil maintain their effectiveness for a greater duration than the traditional aqueous formulation after storage at +4°C and produce positive antibody responses in immunized animals. Moreover, it is safe to administer a booster dosage to animals that had the intramuscular vaccination without causing any local reactions at the injection site (Barnett et al., 1996). An adjuvant, Montanide ISA 206, combined with an inactivated BEF vaccination produced a good, longer-lasting antibody titer for 44 weeks (El-Bagoury et al., 2016). A line of ready-to-disperse, cutting-edge polymeric adjuvants called Montanide gel is intended to increase the safety and effectiveness of aqueous vaccinations. It works by dispersing extremely stable gel particles made of sodium polyacrylate in water. Because of the adsorption qualities of polymers, the depot effect with gradual release enhances the

Correspondence to: mnaziz2017@yahoo.com

recruitment of the innate immune system. With a safety profile comparable to aluminum salts, it offers a notable boost to the immune system. It is simple to spread these readily available adjuvants by gently mixing them. They administer highly stable and fluid vaccinations (Seppic, 2008). In addition to being more immunogenic than aluminum hydroxide, Montanide Gel 01 inactivated RVF vaccine also elicits a long-lasting, fast immunological response, making it a recommended vaccination for emergencies (Abd El-Rahman et al., 2020).

Many industries utilize synthetic compounds called carbomers, which are essentially polyacrylic acid, in their goods because of their ability to suspend, emulsify, and thicken materials. Even at high concentrations up to 100%, carbomers show a minimal potential for sensitization and skin irritation (Rachel Ann Tee-Melegrito, 2022). The enhancing effect of carbomer on the immune response of vaccinated calves with trivalent FMD vaccine adjuvant with carbomer came in agreement with what obtained by Mair et al (2015) reported similar findings with EH-1 and rabies vaccines. In this work we mainly try to provide new improved inactivated PPR vaccine with the best adjuvant that enhance its potency, induce the highest protective immune status in vaccinated sheep.

2. MATERIAL AND METHODS

2.1. Ethical Approval

This research was approved by Institutional Animals Care and Use Committee of faculty of veterinary medicine, Benha university (approved number BUFVTM) 08-01-23)

2.2. PPRV strain and Reference sera

Live attenuated Nigerian strain of PPRV (Nigerian 75/1 strain) adapted on African green monkey kidney cell line (Vero) was provided by The African Union Panafrican Veterinary Vaccine Centre (AU-PANVAC) and maintained by the Department of Rinderpest Vaccine Research (DRVR); Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo and used for preparation of the inactivated PPR vaccine as well as for application of serological tests. The virus had a titer of 6.5 log 10 TC ID_{50} / ml. Reference positive and negative PPR sera were kindly supplied by Department of Rinderpest Vaccine Research to interpretate ELISA results

2.3. Cell line

Vero cell line was kindly provided by the DRVR; VSVRI and utilized in the vaccine preparation and serological tests. This cell line was maintained and passaged using Minimum essential media (MEM) with Hank's salts, L-glutamine and without sodium bicarbonate supplied by Gibco (G80 Gibco Limited, P.O. Box 35 Paisley, Scotland, U.K.) and prepared according to the manufacture directions. The cell culture medium was supplemented with 10% new born calf serum as growth medium while it was used with 3% serum as maintenance medium for cell cultures.

2.4. Adjuvants

Monanide™ Gel-01 as an adjuvant aims to increase the effectiveness of aqueous- type vaccines by acting as a water-based adjuvant of immunity It is a water-soluble dispersion of a synthetic polymer with a low Montanide adjuvant content that falls under the high molecular weight polyacrylic acid category. The adjustment range for Monanide™ Gel (01) is 5–20% (W/W). It was produced by France's SEPPIC. Montanide oil 206 used without the addition of a surfactant ingredient to formulate a water-in oil-in water emulsion or a double emulsion (continuous aqueous phase emulsion inside which droplets of oil contain a secondary aqueous phase). It was acquired from Paris, France's SEPPIC. Carbopol® 940 NF polymer as a fluffy, white powder was provided by Lubrizol Co. In order to create 0.5% aqueous stock solutions, it was dissolved in hot water autoclaved for 20 minutes at 121°C to sterilize it, and then it was kept cold (at 4°C) until needed (United States Pharmacopeial Convention, 1990)

2.5. Virus titration and inactivation

PPR virus was titrated in Vero cell cultures following the method of Burleson et al. (1997) using the micro titer technique and expressed the virus titer as $\log_{10}TCID_{50}$/ml in accordance with Reed and Muench (1938). The PPR virus ($10^{5.5}$ TCID50/ml) was inactivated by 1 M Binary ethylenimine (BEI) in 0.2N NaOH at 37ºC (Ronchi et al., 2016). Complete virus inactivation was confirmed by detection of the residual alive virus titer

2.6. Inactivated PPR Vaccine Formulation

Each of three portions (each portion is 100 ml) of inactivated PPRV was adjuvant with Monanide™ Gel-01 (IPPRV-1) added as 20% (Seppic, 2008) used as aqueous polymeric adjuvant for veterinary vaccines; Montanide ISA oil 206 added as 50/50 (v/v) (IPPRV-2) according to Barnett et al. (1996) and Carbomer at the ratio of 50% according to Naglaa et al. (2020). These vaccine formulae were stored at 5±3ºC until further evaluation (OIE, 2017)

2.7. Sterility test

All of the prepared inactivated prepared PPR vaccine formulae were tested for their freedom from foreign contaminants (aerobic and anaerobic bacteria, fungi and mycoplasma) on specific media according to the recommendation of FAO (1994)

2.8. Safety and Potency tests

Fifty-five healthy native breed sheep were divided into 4 groups as follow: Each of group 1, 2 and 3 included 15 sheep divided into 3 subgroups (A, B, and C including 5 sheep/subgroup) where group-1 vaccinated with the vaccine formula-1; group-2 vaccinated with the vaccine formula-2 and group-3 vaccinated with the vaccine formula-3 in the following manner: Subgroup-A was vaccinated subcutaneously in the neck side with 2ml of the used vaccine formula; subgroup-B vaccinated with 2 doses of the same vaccine with 2 weeks in between and subgroup-C vaccinated with the double dose (4ml) as safety test. Vaccinated sheep groups with IPPR vaccine received 2 doses (2ml/animal) with 2 weeks intervals inoculated SC. Group-4 was kept without any inoculation as test control. All sheep groups were subjected to a daily clinical examination, and were kept in hygienic conditions, and were given a balanced diet and enough water. Using jugular vein punctures, serum samples were taken from all sheep groups every week for up to four weeks and subsequently every month for up to 28 weeks (7 months) following vaccination for monitoring of PPR antibody titers by serum neutralization test (SNT) and indirect enzyme linked immune sorbent assay (ELISA)

2.9. Serum neutralization test (SNT)

SNT was carried out in Vero cell culture using micro-technique method as described by Ferreira (1976) to follow up PPR antibody titers in vaccinated sheep. The neutralizing antibody titers were defined by Singh et al. (1967) as the reciprocal of the final serum dilution that prevented the CPE

2.10. Indirect ELISA

The study employed the combined procedures of Voller et al. (1976) and Hubschle et al. (1981) to perform an indirect enzyme linked immune sorbent test (ELISA) on vaccinated sheep serum samples.

3. RESULTS

The inactivated prepared PPR vaccine appear as an oily whitish pale red; reddish clear and light reddish clear solutions for the forms adjuvanted with Montanide 206 oil, Montanide gel and carbomer respectively (Fig-1).

Fig (1): Inactivated PPR vaccine: 1-adjuvanted with montanide oil 206; 2-adjuvanted with montanide gel; 3-adjuvanted with carbomer

Regarding sterility and safety tests, it was found that the three formulae of the inactivated PPR vaccine that were developed without any external impurities and safe for vaccinated sheep inducing no abnormal local or systemic post vaccinal reactions with normal mean rectal temperature all over a period of 15 days post vaccination (Table-1).

Table (1): Body temperature of experimental sheep groups

Days post vaccination	Sheep groups G1* SGA**	G1* SGB	G2 SGA	G2 SGB	G3 SGA	G3 SGB	G4
0	38	38.4	38.3	39	38.5	38.7	38.5
1DPV***	38.4	39	38	38.4	38.5	38.6	38.3
2DPV	38.6	38.3	38.4	38.3	39	38.7	38
3DPV	38.5	38.7	38.5	38	38.3	38.4	38.5
4DPV	38.5	38.6	38.3	38.6	38.5	38.5	39
5DPV	39	38.7	38	38.5	39	38.6	38.5
6DPV	38	38.4	38.3	39	38.5	38.7	38.5
7DPV	38.4	39	38	38.4	38.5	38.6	38.3
8DPV	38.6	38.3	38.4	38.3	39	38.7	38
9DPV	38.3	39	38.5	38.7	38.5	38	38.3
10DPV	38	38.4	38.5	38.6	38.3	38.6	38.5
11DPV	38.4	38.3	39	38.7	38	38.5	39
12DPV	38.4	38.3	39	38.5	38.7	38.5	38
13DPV	39	38	38.4	38.5	38.6	38.3	38.6
14DPV	38.3	38.4	38.3	39	38.7	38	38.5

*G= Group, **SG= Subgroup,***DPV= days post vaccination, G1: vaccinated with inactivated vaccine adjuvanted with Montanide 206 oil, G2: vaccinated with inactivated vaccine adjuvanted with Montanide gel, G3: vaccinated with inactivated vaccine adjuvanted with carbomer, G4: unvaccinated group, SG: received double doses for safety test

The demonstrated SNT results in table (2) showed that sheep vaccinated with the inactivated PPR vaccine with Carbomer adjuvant exhibited protective antibody titer (8) at the 2nd week after vaccination, whereas those received the vaccine with Montanide 206 oil reached this titer (in SGA) by the 3rd week. It was found that administration of a booster doses (in all SGB) enhanced the induced serum neutralizing antibody titers in vaccinated sheep to be 32 in all SGB by the 3rd Week post the first vaccination (1week post booster). The titers reached their maximum value of (128) at the second month post vaccination that still unchanged up to 7 months (the experimental period). ELISA results were interpreted by reference control and the positive antibody titer was expressed as log10 (titer less than 0.5 is considered negative), Table (3)

Table (2): Mean PPR serum neutralizing antibody titer

WPV***	Sheep groups G*-1 SG**A	G-1 SGB	G-2 SGA	G-2 SGB	G-3 SGA	G-3 SGB	G-4
0	0	0	0	0	0	0	0
1WPV	2≤	2≤	2≤	2≤	2	2	0
2WPV	4	4	4	4	8	8	0
3WPV	16	32	16≤	32	16	32	0
4WPV	32	64	32	64	32	64	0
2MPV ****	←128→ Remained constant up to 7MPV						0

*G= Group,**SG= Subgroup,***WPV= week post vaccination**,******MPV= month post vaccination, Subgroups A received one vaccine dose; subgroups B received 2 vaccine doses with 2 weeks in between; and group-4 kept without vaccination ,*Serum neutralizing antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of PPR virus; SNT titer >=8 deemed to be protective (*Santhosh et al., 2013)*

Table (3): PPR -indirect ELISA titer (log10/ml)

WPV***	Sheep groups G*-1 SG**A	G-1 SGB	G-2 SGA	G-2 SGB	G-3 SGA	G-3 SGB	G-4
0	0.02	0.03	0.03	0.04	0.10	0.02	0.04
1WPV	0.50	0.5	052	0.51	0.60	0.60	0.03
2WPV	0.71	0.72	0.72	0.70	0.75	0.76	0.02
3WPV	1.01	1.50	1.20	1.52	1.55	1.60	0.04
4WPV	2.40	2.50	2.30	2.70	2.30	2.71	0.03
2MPV ****	←2.3-2.5→						0.03

*G= Group, **SG= Subgroup,***WPV= week post vaccination,****MPV= month post vaccination,Subgroups A received one vaccine dose; subgroups B received 2 vaccine doses with 2 weeks in between; and group-4 kept without vaccination

4. DISCUSSION

Over the course of the last two decades, PPR has spread significantly throughout Africa and Asia, affecting previously uninfected nations. Due to direct losses from mortality and decreased productivity in afflicted animals, the introduction of PPR into a region that is free of the disease (such as Europe) would have a significant detrimental economic impact. One of the best ways to reduce losses brought on by virus transmission, both direct and indirect, is vaccination. A control program requires a potent vaccine against PPR (Singh, 2011). Live vaccines are the only available product on the market right now. In this regard, vaccines containing entire inactivated viruses might be a safer substitute. In our current study, we assessed the effectiveness of a novel inactivated adjuvanted vaccine (PPR vaccine) using three different adjuvants. According to Ronchi et al. (2016), inactivated PPR is a potentially effective substitute for live attenuated vaccines in PPR immunization programs conducted outside of endemic areas. When choosing or developing adjuvants for animal vaccines, a number of factors should be taken into account: efficacy in the target animal species; induction of a prompt and durable protective immunity; animal safety; adherence to food safety regulations; feasibility of scaling up production; and, last but not least, cost effectiveness (Burakova et al., 2018).The results acquired thus far indicate that the three created PPR vaccine formulae were deemed safe and effective, as reported by Ghattas et al. (2007), who determined that the PPR vaccine is safe and causes highly

satisfactory serological responses in vaccinated sheep. In rats and goats, the injection of binary ethyleneimine-inactivated PPR virus elicited humoral responses and was safe (Ronchi et al., 2016). This inactivated PPRV vaccine formulated with Montanide oil protects the natural host against homologous virus challenge. These results demonstrated that the PPR inactivated vaccine formulation using Montanide 206 oil and Montanide gel produces a similar and efficient specific immunological response in vaccinated sheep as previously shown by Rodríguez-Mallon et al. (2020) and Pedro et al. (2021) who found that both adjuvants induce similar immune response in pigs. Each formula of Montanide 206 oil and Carbomer adjuvanted PPR vaccine was demonstrated to be devoid of extraneous impurities, safe, and effective exhibiting no post-vaccinal responses and higher protective concentrations of specific PPR antibodies as represented by Walaa and Abeer (2021) who found that FMD antibodies were with high levels in vaccinated Guinea pigs with these adjuvants vaccine formulae. Because of their composition, Montanide ISA for W/O/W emulsions can activate immune responses that are both humoral and cell-mediated. Recombinant and inactivated antigens can be used with SEPPIC W/O/W adjuvants. W/O/W emulsion has a high fluidity and is well tolerated. Vaccines adjuvanted with Montanide ISA 206 oil show no signs of toxicity or prolonged pyrexia after administration, and cattle vaccinated intramuscularly do not show any local reactions at the site of inoculation. These vaccines also maintain their potency longer than the conventional aqueous formulation (Barnett et al., 1996). It was concluded that the combination of the Montanide ISA 206 adjuvant with the inactivated BEF vaccine produced a good higher and longer-lasting antibody titer for 44 (WPV) week post vaccination. (El-Bagoury et al., 2016). Adjuvants like Montanide gel can be used with a variety of antigenic media and are advised for parenteral or mucosal delivery of live or inactivated vaccines. They give fluid and very stable vaccines (Seppic, 2008). It was found to be highly immunogenic than Aluminum Hydroxide beside and produces a long-lasting, fast-onset immune response that is advised for emergency immunization (Abd El-Rahman et al., 2020). Since carbomers (as synthetic ingredient) have the ability to suspend, emulsify, and thicken materials, several industries use them in their goods. Insoluble materials that do not dissolve into liquid are helped to suspend and disperse by carbomers. Additionally, they stop a formulation's liquid and oil components from separating. even at high carbomer concentrations of up to 100%, there is a limited possible risk of hypersensitivity and skin irritation associated with carbomers (Rachel Ann Tee-Melegrito, 2022). The findings by Mair et al. (2015) on the rabies and EH-1 vaccines were consistent with the boosting effect of carbomer on the immunological response of calves vaccinated with trivalent FMD vaccine adjuvanted with carbomers. The results of comparing three adjuvants showed that the average PPR antibody titers induced by both of them had no statistically significant difference.

5. CONCLUSION

From the obtained results it could be concluded that The prepared inactivated PPR vaccine is a safe and potent vaccine able to induce protective immunity in vaccinated sheep and each of Montanide ISA; Montanide gel and Carbomer has safely the ability to adjuvant the inactivated PPR.

CONFLICT OF INTEREST

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

6. REFERENCES

1. Abd El Rahman, S.E., Abul Magd, D. M., Atwa, M. H. & Soliman, S. M., 2020. Evaluation of The Cellular and Humoral Immune Response of Sheep Vaccinated with Inactivated Rift Valley Fever Vaccine Adjuvanted with Montanide Gel 01TM. Journal of Applied Veterinary Sciences, 5,1 :22-34.
2. Ahmed, S., Hosny, W.A., Mahmoud, M., Mahmoud, M.A., 2021. Isolation and identification of peste des petits ruminant's virus from goats in Egyptian governorates. Veterinary World, 14,4 : 926-932.
3. Bahnemann, H.G., 1990. Inactivation of viral antigens for vaccine preparation with particular reference to the application of binary ethylene. Virus disease, 2014 Jan., 25,1 : 39–56.
4. Barnett, P., Pullen, L., Williams, L. & Doel, T.R., 1996. Assessment of Montanide ISA 25 and ISA 206, two commercially available oil adjuvant. Vaccine, 14,13 : 1187-1198.
5. Burakova, Y., Madera, R., McVey, S., Schlup, J. R., and Shi, J. 2018. Adjuvants for Animal Vaccines. Viral Immunol. 31, 11–22.
6. Burleson, F.G., Chambers, T.M. and Wiedbrank, D.L., 1997. Virology: a laboratory manual. Academic Press/Harcourt., Brace Jovanovich, New York, 216-8233-2.
7. Daoud, H.M., Ibrahim E.E., El-Din, W.M.G. and Hassanin, A.I.H., 2013. Preparation of foot and mouth disease trivalent vaccine type A, O, SAT2 and determination of the Guinea pig protective dose 50 ,GPPD50 . Vet. World, 6,11 :844–851.
8. El-Bagoury, G. F., El-Habbaa A. S., Amal A. M., and Nermeen G. S. 2016. Efficacy of inactivated BEF vaccine adjuvant with Montanide ISA 206 compared with the locally prepared inactivated BEF vaccines. Benha Vet. Med. J., vol. 31 issue 2, pp:117-123.
9. FAO, 1994. Quality control of rinderpest cell culture vaccine. Standard Operating Procedures, FAO report No.118.
10. Ferreira, M. E., 1976. Prubada microneutralization proesludaes de anticurpos de la fiebre aftosa blin Centro. Pan-American de ubrea Akrosa, 211: 17 – 24.
11. Food and Agriculture Organization of the United Nations ,FAO /World Organization for animal Health ,OIE , 2015. Global strategy for the control and eradication of ppr– GF-TADs FAO. https://www.fao.org /3/a-ak136e.pdf and OIE https://www.oie.int/en/for-the-media/press-releases/detail/article/new-initiatives-by-the-oie-and-its-partners-to-improve-animaland-public-health-in-africa/.
12. Foumani, M., Asadpour L., Azizi Saraji A., Sharifat Salmani A. and Aghasadeghi M., 2012. Adjuvants and their mechanisms of action. J. Ardabil Univ. Med. Sci., 12: 276–291.
13. Ghattas, W.M., Hussein, G., Sayed, Fatma., Abdel Azeem, Laila., Taha, Samira S. and Ayad, Samia A., 2007. Immunological studies on simultaneous vaccination with pneumo-3 and ppr inactivated vaccine in sheep. Assiut veterinary medical journal, v. 53 ,112 1-12.
14. Hubschle, O.J., Lorent, R.J. and Mathok, H.D., 1981. Enzyme linked immune-sorbent assay for detection of blue tongue virus antigen. Am. J. Vet. Res., 42: 61-65.
15. Ibrahim, E.E., 2011. Advanced studies on Foot and Mouth Disease Vaccines of sheep in Egypt. Thesis, Ph.D. Sc. ,Infectious Diseases , Faculty of Veterinary Medicine, Cairo University, Egypt.
16. Ikram, A.K., El-Danaf, N.A., El-Hakashly, S. and House, J. ,1988 . Isolation of a viral agent from Egyptian goats suspected to be PPR-virus. J. Egypt. Vet. Med. Ass., 48,3 :429-435.
17. Jin, Z., Gao S., Cui, X., Sun D. and Zhao, K., 2019. Adjuvants and delivery systems based on polymeric nanoparticles for mucosal vaccines. Int. J. Pharm. 572:118731. Doi: 10.1016/j.ijpharm.2019.118731.
18. Maged, A., Nafea R., Elbakry, M., Shahein, M., Gamelat, K. Farag, Fatma, Abdallah, Ahmed, A.H. Ali, 2019. Virological and Molecular Studies on Peste des Petits Ruminants Virus (PPRV) in Small Ruminants and Camel in Egypt between 2017

and 2018; Advances in Animal and Veterinary Sciences 2019; Volume Page 12-18.

19. Mair, K.H., Koinig, H., Gerner, W., Höhne, A., Bretthauer, J., Kroll, J.J., Roof, M.B., Saalmüller, A., Stadler, K. and Libanova, R., 2015. Carbopol improves the early cellular immune responses induced by the modified-life vaccine Ingelvac PRRS® MLV. Veterinary Microbiology, 176, 3–4, pp.352-357.
20. Meeusen, E.N.T., Walker, J., Peters, A., Pastoret, P. P. and Jungersen, G., 2007. Current status of veterinary vaccines. Clin. Microbiol. Rev. 20,3 , 489–510. https://doi.org/10.1128/CMR.00005-07.
21. Naglaa, I. Aly., Omaima, A. El-Shamandy., Shendy, M.B., Fatma, F. Warda. & Ekbal, M. Farouk., 2020. Efficacy of using Carbopol as an adjuvant for tissue culture inactivated rabies vaccine. Journal of Applied Veterinary Sciences, 5 ,3 103-107.
22. OIE., 2017. Infection with Pest des Petits ruminant's virus. Terrestrial Animal Health Code. OIE, Paris, 2017.
23. Pedro, E. E. G., Anayram, P. M., Yamil, B. S., Frank L. L. B., William, F. H., Carlos, P. H., Llilian, G. P., Nemecio, G., Mario, P. E. and Alina R.-M., 2021. Comparison of Montanide™ GEL 01 and oily MontanideTM ISA 50 in presenting a peptide to the immune system of dogs. Journal of Veterinary Medicine and Animal Health 13,1 , 28-33.
24. Rachel Ann Tee-Melegrito, 2022. What are Carbomers. Vet. Med. Manuscript, https://www.medicalnewstoday.com /articles/carbomer.
25. Razmaraii, N., Toroghi, N., Babaei, H., Khalili, I., Sadigh, E.S. and Froghy L. 2012. Immunogenicity of commercial, formaldehyde and binary ethylenimine inactivated Newcastle disease virus vaccines in specific pathogen free chickens. Arch Razi Inst. 2012, 67, 21-5.
26. Reed, L.J. and Muench, H., 1938. A simple method for estimating fifty percent ,50% end points. Amer. J. Hyg. 27, 493-497
27. Reed, S. G., Orr, M. T. and Fox, C. B., 2013. Key roles of adjuvants in modern vaccines. Nature Medicine 19, 1597-1608.
28. Rodríguez-Mallon, A., Gonzalez, L.J., Encinosa Guzman, P.E., Bechara, G.H., Sanches, G.S., Pousa, S., Cabrera, G., Cabrales, A., Garay, H., Mejias, R. et al., 2020. Functional and mass spectrometric evaluation of an anti-tick antigen based on the P0 peptide conjugated to Bm86 protein. Pathogens, 9,6 . https://doi.org/10.3390/pathogens 9060513
29. Ronchi, G.F., Monaco, F., Harrak, M., Chafiqa, L., Capista, S., Bortone, G., Orsini, G., Pinoni, C., Iorio, M., Iapaolo, F. et al., 2016. Preliminary results on innocuity and immunogenicity of an inactivated vaccine against Pest des petits ruminants. Vet Ital. j. 52,101-9.
30. Santhosh, A.K., Gomes, A.R., Hegde, R., Rathnamma, D., Veeregowda, B.M., Byregowda, S. M., Renukaprasad, C., Bhanuprakash, V., Prabhudas, K., Hegde, N.R., et al., 2013. Comparative immunogenicity of two Peste des Petits Ruminants ,PPR vaccines in South Indian sheep and goats under field conditions. Indian J. Virol. 24,3 : 373–379. http://dx.doi.org /10.1007/s13337-013-0168-6.
31. Seppic, 2008. Technical Bulletin Montanide TM01 gel ready to use aqueous polymeric adjuvant for veterinary vaccines. www.seppic.Com.
32. Singh, K.V., Osman, O.A., Thanaa, I. Baz and Ivon El-Cicy 1967. Colostrum transfer of rinderpest neutralizing antibodies to offspring of vaccinated dams. Can. J. Comp. Med. Vet. Sci., 31: 295-298.
33. Singh, R.P., 2011. Control strategies for peste des petits ruminants in small ruminants of India. Rev. Sci. Tech. Epiz. 2011, 30, 879–887. https://doi.org/ 10.20506/rst.30.3.2079.
34. Verma, S. K., Mahajan, P., Singh, N. K., Gupta, A., Aggarwal, R., Rappuoli R., and Johri A. K., 2023. New-age vaccine adjuvants, their development, and future perspective. Front. Immunol. Sec. Vaccines and Molecular Therapeutics, 14-2023. https://doi.org/ 10.3389/ fimmu.2023. 1043109.
35. Voller, A., Bidwell, D., and Bartlett, A., 1976. Enzyme immunosorbent assay for diagnosis of virus infection. Manual of clinical Microbiol, Washington, D.C. Am. Soc. Microbiol.,136, 506-512.
36. Walaa, S. Shabana and Abeer, Abd EL-Sadek, 2021. Effect of Carbomer as an Adjuvant for Enhancement of Immune-Response Against FMD Vaccine. JAVS., 6 ,2 : 27-36. DOI:10.21608 / JAVS.2021.154578