**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 (Ibrahim 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. (Meusen 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 (Bannhemann, 1990 and Razmariai 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 poylacrylate in water. Because of the adsorption qualities of polymers, the depot effect with gradual release enhances the
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 carboxomers, 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%, carboxomers show a minimal potential for sensitization and skin irritation (Rachel Ann Tee-Melegrito, 2022). The enhancing effect of carboxomer 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 BUVFVTM) 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), Abbassa, 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 TCID 50 / ml. Reference positive and negative PPR sera were kindly supplied by Department of Rinderpest Vaccine Research to interpret 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 37°C (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 Nagla et al. (2020). These vaccine formulae were stored at 5±3°C 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 IPPRV 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).

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

<table>
<thead>
<tr>
<th>Days post vaccinatio n</th>
<th>Sheep groups</th>
<th>G1*</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>SGA A</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>1DPV***</td>
<td>SGA B</td>
<td>39</td>
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<td>38</td>
<td>38</td>
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<td>SGA A</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>3DPV</td>
<td>SGA B</td>
<td>39</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>4DPV</td>
<td>SGA A</td>
<td>39</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>5DPV</td>
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<td>39</td>
<td>38</td>
<td>38</td>
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</tr>
<tr>
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<td>SGA A</td>
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</tr>
<tr>
<td>7DPV</td>
<td>SGA B</td>
<td>39</td>
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<td>38</td>
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</tr>
<tr>
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<td>SGA A</td>
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<td>38</td>
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</tr>
<tr>
<td>9DPV</td>
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<td>38</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>SGA B</td>
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<td>38</td>
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<tr>
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<td>SGA A</td>
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<td>14DPV</td>
<td>SGA A</td>
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<td>38</td>
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<td>38</td>
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</tbody>
</table>

*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 (1 week 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

<table>
<thead>
<tr>
<th>WPV</th>
<th>G*1</th>
<th>SGB A</th>
<th>SGB B</th>
<th>SGB A</th>
<th>SGB B</th>
<th>SGB A</th>
<th>SGB B</th>
<th>SGB A</th>
<th>SGB B</th>
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</thead>
<tbody>
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<td>0.03</td>
<td>0.03</td>
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<td>0.10</td>
<td>0.02</td>
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<tr>
<td>1WPV</td>
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<td>0.52</td>
<td>0.52</td>
<td>0.51</td>
<td>0.60</td>
<td>0.60</td>
<td>0.03</td>
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<tr>
<td>2WPV</td>
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<td>0.72</td>
<td>0.72</td>
<td>0.70</td>
<td>0.75</td>
<td>0.76</td>
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<tr>
<td>3WPV</td>
<td>1.01</td>
<td>1.50</td>
<td>1.20</td>
<td>1.52</td>
<td>1.55</td>
<td>1.60</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4WPV</td>
<td>2.40</td>
<td>2.30</td>
<td>2.30</td>
<td>2.70</td>
<td>2.30</td>
<td>2.71</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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 formulate were deemed safe and effective, as reported by Ghaffar 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–Mallón 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 Walaal 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 (Seppe, 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 and Additi, 2011). The authors declare that they have no conflicts of interest for current data

6. REFERENCES


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