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Potency of oil adjuvant inactivated Peste des Petits Ruminants (PPR) vaccine

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ABSTRACT

The Peste des petits ruminant (PPR) is a viral disease caused by a morbilli virus resulted in huge economic losses among sheep and goats. Vaccination is the essential corner stone to control it. Preparation of a safe potent inactivated vaccine could be used during outbreaks or in enharbouring countries free from the disease. Preparation of PPR virus infected fluid by virus inoculation on Vero cell line followed by virus titration and inactivation by binary ethylene amine and adjuvant by Montanide oil ISA 206 followed by application of quality control testing on the final preparation (freedom from foreign contaminants; safety and potency in susceptible sheep). The prepared inactivated PPR vaccine adjuvanted with Montanide 206 oil is safe and potent able to provide vaccinated sheep with high protective levels of specific immunity. The inactivated PPR vaccine can be used for immunization of sheep protecting them against the virus infection especially those received 2 doses on 2 weeks interval. It is suggested that the obtained immunity may be of long duration remain up to 12 months post vaccination.

1. INTRODUCTION

The Peste des petits ruminant (PPR) is known as a virulent widespread, devastating disease of small ruminants (FAO and OIE, 2015). PPR virus; the causative agent; is a member of the morbilliviruses which display a strong lymphoid tissue tropism and destruction of leucocytes causing a profound immunosuppression (Rajak et al., 2005). It causes severe clinical signs depending on the species, age, strain virulence and secondary infectious agents (Zahur et al, 2009; Kivaria et al, 2013; OIE, 2013 and Chowdhury et al, 2014). Such signs include pyrexia, respiratory symptoms, immunosuppression, and despondency, and erosive stomatitis, catarrhal inflammation of ocular and nasal mucus, profuse watery fetid and bloody diarrhea and frequently end-stage bronchopneumonia. PPR virus is closely related morbillivirus, much like rinderpest (OIE, 2000). The disease was first identified in West Africa and is currently found in the Middle East, sub-Saharan Africa, and the Asian subcontinent, which includes Bangladesh, Nepal, and Tibet. There has been speculation that this virus has recently spread to regions where the rinderpest virus was eliminated (Baron et al., 2016).

Mainly the corner stone for control of infectious diseases like PPR depends on the use of live-attenuated vaccines which in the endemic area is a primary controlling method On the other hand, inactivated PPR vaccination could be advised throughout the PPR eradication procedure (Akbarian et al., 2021). Inactivated vaccines need adjuvants to initiate their potency. They are ingredients used in inactivated vaccines to create a strong immune response in hosts receiving the vaccine where they help vaccines work better in addition to help the body to produce strong an immune response enough to protect the animals from the disease they are being vaccinated against. The use of Montanide oil 206 (Seppic, Paris) indicated that vaccines adjuvanted with it induced strong antibody responses in pigs and cattle and such vaccines remained potent longer than those adjuvanted with other adjuvants after storage at +4°C. It also showed no signs of toxicity or extended pyrexia after delivery. Cattle that received an intramuscular vaccination showed no signs of local responses at the injection site. It was suggested that this oil have potential as an alternative to other aqueous formulation (Barnett et al.,1996). Montanide adjuvant is experimental adjuvant designed as an emulsion of water-in-oil or water-in-oil-in-water, which has been shown to cause high antibody titers in a number of animal species (Miles et al., 2005).The present study aims to prepared and evaluate the potency of an oil adjuvant inactivated PPR vaccine in 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

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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. Preparation of Montanide-Adjuvanted inactivated PPR vaccine

2.4.1. Virus propagation and titration

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).

2.4.2. Inactivated PPR Vaccine Formulation

PPR virus ($10^{6.5}$ $TCID_{50}$/ml) was inactivated by the addition of 1 M Binary Ethylenimine (BEI) in 0.2N NaOH to the viral solution, resulting in a final BEI concentration of 0.01 M. The mixtures of virus and BEI were thoroughly mixed, then sodium bicarbonate was added, to adjust the pH to 8.0. The virus was incubated for 12 hours at 37°C on a magnetic stirrer. Briefly, the PPR virus inactivation kinetics was ascertained by subjecting it to 1 mM BEI at various post-inactivation times at 37 °C (Ronchi et al., 2016). Serial sampling and residual alive virus titration at 60-minute intervals were used to assess the level of virus inactivation. After the inactivation period, sodium thiosulfate (Sigma-Aldrich, U.S.A.) was used to neutralize the BEI after each sampling at a final concentration of 2% (v/v). The microtitration method was used to measure the PPRV titer at each time interval. The inactivated PPR vaccine was prepared in accordance with Fayed et al. (2000) and Hussein (2001). The inactivated virus was mixed with the Montanide oil ISA 206 at a volumetric ratio of 1:1 (Aslam et al., 2013)

2.5. Sterility, safety and stability test

Five random samples of the prepared vaccine were cultured on specific media: Saburaoud glucose agar, which looked for fungal contamination after incubation at 25°C for 14 days; Nutrient agar media; and Thioglycolate broth, which looked for aerobic and anaerobic bacterial contamination, respectively, after incubation at 37°C for 72 hours in addition to mycoplasma detection on specific mycoplasma medium. Testing the safety of the prepared inactivated oil PPR vaccine was carried out in accordance with Akbarian et al (2021), the inactivated virus suspension was tested for any active viruses by growing on the Vero cell after the inactivation process. In addition, toxicity tests were carried out in compliance with the European Pharmacopeia monograph. Twenty Swiss Albino mice (17-22 g) were randomly divided into two groups (10 animals each) and received 0.1 ml of the prepared vaccine intraperitoneally (IP), with all animals monitored for any signs of illness over a 14-day period. The stability of the prepared vaccine carried out according to Abaracon et al (1982), sample from the prepared vaccine was centrifuged at highspeed cool centrifuge at 10000 rpm for 60 minutes to determine the oil stability. In addition, vaccine samples were kept at 4°C and examined for their potency every 6 months up to 24 months post preparation

2.6. Experiment for Potency testing

Twenty-five native breed sheep (aged 6-12 months) free of external and internal parasites and with serum negative for PPR antibodies (as shown by a serum neutralization test) were divided into 3 groups as follow: Group-1 of 10 sheep inoculated subcutaneously with one dose of the prepared vaccine (2ml) according to Fayed et al. (2000). Group-2 of 10 sheep inoculated subcutaneously with 2 vaccine doses with 2 weeks interval. Group-3 of 5 animals was kept without vaccination. All sheep groups were housed under sanitary conditions, with balanced rations and appropriate water. Serum samples were collected from all sheep at weekly and monthly intervals up to 12 months post vaccination for monitoring the induced PPR immune status by serum neutralization test (SNT) and indirect enzyme linked immune sorbent assay (ELISA)

2.7. 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

2.8. 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

Subjection of PPR virus to the inactivation process, revealed that complete virus inactivation was after 5 hours of processing (Fig 1) as detected by inoculation on Vero cell line showing no CPE. Testing the freedom of the prepared vaccine showed that it is free from foreign contaminants (aerobic and anaerobic bacteria, fungi and mycoplasma). Inoculation of mice with the inactivated PPR vaccine did not show any local or systemic post inoculation abnormal signs indicating its safety. Also, the vaccine safety was observed in vaccinated sheep where they remained with normal body temperature (38.5-39.0^{O}C) all over 15 days post vaccination and normal general health condition.

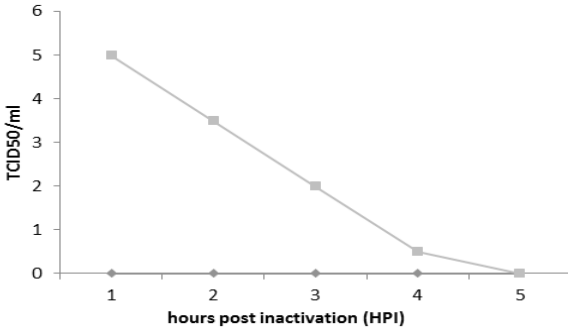

Fig (1): Inactivation rate of PPR virus by binary ethyleinemine

Monitoring of the levels of induced immunity in vaccinated sheep through application of SNT showed that all vaccinated sheep exhibited protective levels of specific serum neutralizing PPR antibodies (8 & 16) by the 3rd week reaching their peak (128) by the 8th week post vaccination in both sheep groups receiving 1 and 2 doses and persisted up to 28 weeks post vaccination as shown in table (1).

Table (1): Mean PPR serum neutralizing antibody titer

Week Post Vaccination (WPV)	Mean PPR serum neutralizing antibody titer/sheep group		
	Group1	Group2	Group3
Pre-vaccination	0	0	0
1WPV	2<	2<	0
2WPV	4	4	0
3WPV	8	16	0
4WPV	32	64	0
8WPV	128	128	0
12WPV till 28 WPV	128	128	0

Group-1: vaccinated with one dose, Group-2: vaccinated with 2 doses (booster dose on the 2nd WPV).,Group-3 Unvaccinated control, Serum neutralizing antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of $100TCID_{50}$ of PPR virus; SNT titer >=8 deemed to be protective (*Santhosh et al., 2013)*

ELISA results showed similar manner as those of SNT revealing that sheep vaccinated with 2 doses exhibited higher levels of antibodies (2.0 log10) than those vaccinated with one dose (1.8 log10)) by the 3rd week post vaccination with peak titers 3.5 and 3.2 log10 by the 8th week remaining with high protective levels (3.5-3.65 log10) up to 28 weeks. These results are tabulated and demonstrated in table (2)

Table (2): Mean PPR ELISA antibody titer

Week Post Vaccination (WPV)	Mean PPR ELISA antibody titer/sheep group		
	Group1	Group2	Group3
Pre-vaccination	0	0	0
1WPV	0.71	0.70	0
2WPV	1.0	0.9	0
3WPV	1.8	2.0	0
4WPV	2.1	2.5	0
8WPV	3.2	3.5	0
12WPV till 28 WPV	3.5-3.6	3.5-3.65	0

Group-1: vaccinated with one dose, Group-2: vaccinated with 2 doses (booster dose on the 2nd WPV). Group-3 Unvaccinated control, ELISA results were interpreted by reference control and the positive antibody titer was expressed as log10

4. DISCUSSION

PPR, among the most economically significant diseases of small ruminants, continues to kill millions of goats and sheep each year throughout many African, Middle Eastern, and Asian nations. Vaccination is still the most efficient method of disease control in endemic areas (FAO/OIE, 2015). Although a live PPR vaccine is highly successful at controlling the disease and providing long-term protection, (Saravanan et al., 2010 and Hodgson et al., 2018), it is highly sensitive to temperature and must be kept and distributed carefully within an efficient cold chain. Inactivated vaccines have several advantages in terms of safety and stability, and they can be used in non-endemic countries where live vaccinations are not approved by veterinary authorities. So, we prepared and evaluated the safety and potency of an inactivated PPR vaccine using Nigerian 75/1 strain adapted to Vero cell line. The determined time to reach complete inactivation of 6.5log10 $TCID_{50}$ of PPR virus using BEI was found to be 5 hours (table-1 and fig-1) coming parallel to that determined by Akbarian et al. (2021) who demonstrated that it took around 6 hours to achieve total viral inactivation with titer decrease of 1.5 log10 $TCID_{50}$/hour. Such virus inactivation with BEI was a linear reaction as concluded by Bamouh et al. (2023) but differs from their determined inactivation time which was 7 hours where they used higher virus titer utilizing Binary Ethyleneimine (BEI) at a concentration of 1 mM at 37°C. The prepared inactivated PPR vaccine was found to be free from foreign contaminants and safe induced no local or systemic abnormal post vaccinal reactions in accordance with Fayed et al. (2000); Hussein (2001); Akbarian et al. (2021) and Bamouh et al. (2023) who concluded that inactivated vaccine may be a beneficial tool for PPR prevention since they eliminate the thermos-sensitivity issues associated with live immunizations. Evaluating the potency of the prepared inactivated PPR vaccine, it was found that the results of SNT and ELISA demonstrated in tables (2&3) and fig (2&3) revealed that it is potent vaccine providing vaccinated sheep with protective antibody levels by the 3rd week post vaccination with recorded peaks by the 8th week and still without decline up to 28 weeks later. The PPR antibody titers obtained appear to be greater above the suggested values, with SNT titers >=8 considered protective (Santhosh et al., 2013). Also, our results came to be supported by the findings of Ronchi et al. (2016) stated that an adjuvanted inactivated PPR vaccine produced 100% seroconversion rate in immunized goats at day 9 and was still positive for 4 months, and Cosseddu et al. (2016) also developed an adjuvanted inactivated PPR vaccine which caused 100% seroconversion rate in immunized goats that resisted the virulent challenge. In addition, an oil-based adjuvanted PPR vaccine employing Oil-in-Water (O/W) emulsion, which is known to be safe and boost humoral immunity (Schijns et al., 2014 and Tahara et al., 2022). It has been shown that oily adjuvanted vaccines induce longer lasting protection against viruses than watery preparations (Hamdi et al., 2020 and Es-Sadeqy et al., 2021). Our results indicated that administration of one dose, induced antibody response, at 1week post vaccination with higher levels with the use of two doses as demonstrated by Couacy-Hymann et al. (2007) and Shaila et al. (1989) who determined a 100% seroconversion rate at day by the 2nd week using one dose. Seeing a substantial difference between the two tested doses for the first 4 months, and then antibody titers become identical, they concluded that the necessity for a booster injection four weeks after the first treatment could be a constraint in eliciting long-term immunity. In addition, it was stated that in rats and goats, inoculation with binary ethyleneimine inactivated PPR virus was safe and elicited humoral responses. Seroconversion was produced in goats from day 9 to day 30 post-first immunization, which was transformed into a robust and durable PPRV neutralizing antibody response after a booster immunization (day 36) until at least day 110 post-booster. The natural host is protected against homologous viral challenge by this inactivated PPRV vaccination. After two injections, the IPPRV formulation with Montanide oil adjuvant caused 100% seroconversion in rats, and goats immunized twice subcutaneously, 36 days apart, seroconverted to PPR by day 9 and remained seropositive until the conclusion of the trial period (133 days). For PPR immunization efforts in non-endemic areas, these data were found to offer a promising substitute for live attenuated vaccines (Ronchiet al., 2016).

5. CONCLUSIONS

In conclusion, this research provides proof that the prepared inactivated PPR vaccine elicited high level of neutralizing antibodies against PPRV and provided full protection against a highly pathogenic PPRV infection. Immunity from the vaccine is maintained for at least a year, and an annual booster shot is necessary. Furthermore, there is no risk of pathogenicity, shedding, or the spread of foreign agents in small ruminants when administered the vaccination. The vaccine is a viable option to stop the spread of PPRV in the at-risk nations, and it could be a useful tool to use in the worldwide PPR eradication program that combines vaccines.

CONFLICT OF INTEREST

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

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