Pak. j. life soc. Sci. (2016), 14(3): 178-182 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

RESEARCH ARTICLE

Experimental Trials of Foot-and-Mouth Disease Vaccines with Different Vegetable Oil Adjuvants in Goats under Field Conditions

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ARTICLE INFO

Received: Jul 20, 2016
Accepted: Dec 19, 2016
Online: Dec 21, 2016

Keywords

Aluminium hydroxide and saponin
Foot-and-mouth disease
Indirect haemagglutination assay
Olive oil
Peanut oil
Sunflower oil

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ABSTRACT

The study was planned for assessing the effect of vegetable oils (peanut, olive oil and sunflower) as adjuvants in foot and mouth disease monovalent vaccines. Total 30 goats were randomly selected and distributed into 5 groups A to E with 6 animals in each group. Group A was vaccinated with aluminium hydroxide and saponin adjuvanted commercial vaccine. Group B, C and D were vaccinated with peanut, sunflower and olive oil adjuvanted vaccines, respectively. Group E being unvaccinated served as negative control. A booster dosage was administered on 21st day after inoculation. Serum samples were collected at regular intervals following initial vaccination and antibody titers were measured by indirect haemagglutination assay (IHA). After initial immunization the highest titer was found in group A than any of other group. The increased titer of group A was not statistical significant ($P>0.05$) when compared to group B and C. Group D showed nonsignificant ($P<0.05$) titers after initial vaccination. After booster dosage, the rise in titer in B, C and D was significantly higher as compared to group A. Geometric mean titers of all vaccinated groups (A to D) were not significantly different ($P>0.05$) from one another. The present study recommends the use of various locally available, cheaper oils as vaccine adjuvant for effective and comparable results to that of expensively adjuvanted vaccines.

INTRODUCTION

Foot and mouth disease (FMD) is acutely infectious malady of domestic and zoo animals belonging to the order artiodactyla (Bastos et al., 2003). The disease is manifested in the form of vesicles on feet, teats and mouth of affected animals. Moreover, it causes major economic damage to farmers by decreased production of milk, weight loss and abortions. FMD being the OIE list A disease globally treated as unacceptable in trading animals and their products (James and Rushton, 2002).

This causative agent of FMD is an *Aphthovirus* of family *Picornaviridae* (Bablanian and Grubman, 1993). This is a RNA virus and its genome mass is about 8.5 kbs with icosahedral capsid. It possesses four structural proteins among these VP1 plays a key role in immunogenesis (Cloette et al., 2008).

In FMD free countries strict control is lifted on movement and trade of animals. Diseased and exposed animals are usually quarantined and slaughtered (John, 2002). In countries like Pakistan wherever the infectious agent is prevalent, immunization of the animals remains the only effective way for the disease control (Orsel et al., 2007).

Aluminum hydroxide along with saponin (AS) is presently one of the commonest adjuvants used in a myriad of animal diseases. Oils are also used as adjuvant and are known for superior defense as compare to Alum adjuvanted preparations (Sadir et al., 1988). The major drawback related using the oil adjuvanted preparations is confined tissue responses

such as cyst and granuloma development. One of the major reasons of such response is the scums found in oil. Thus it is recommended to use oils which are less sticky with hydrocarbon chain size of 13-29 (Bomford, 1977). Using hydrocarbons with reduced chain length is well known for causing swelling of inoculated tissue (Gupta et al., 1993).

Currently, a variety of herbal oils is being used as adjuvants in vaccines. The major benefit of using plant oils than inorganic oils is the biocompatibility. Plant oil adjuvants make it possible to boost up the immune response of animals against the injected antigen (Gupta et al., 1993). Recent research showed that maize, peanut, soya bean, rice, olive, sesame, linseed, canola, rapeseed and cashew nuts can elicit an active immune response against antigen (Barnett et al., 1996).

Thus, current study was designed with the aims of preparation and relative assessment of different locally available cheaper plant oil as adjuvant of FMD vaccines and trailed in beetal goats. The efficacy of different plant oil adjuvanted vaccine was checked in relations of post-shot local and systemic humoral immune responses.

MATERIALS AND METHODS

Preparation of FMD serotype 'O' vaccines

FMD virus was obtained from University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. The virus titer was calculated and tissue culture infectious dose ($TCID_{50}$) was fixed at 10^6 units. Inactivation of antigen was done by Binary etyhyleneimine (BEI) (Bahnemann, 1975). Plant oils were used as adjuvants of vaccine preparations along with surfactants as follows: i) Peanut oil/Sunflower Oil/Olive Oil (100 mL), ii) Span-80 (8.3 mL), iii) Tween-80 (2.7mL) and iv) Viral suspension (Serotype O; 25 mL).

Both plant oils and surfactants were subjected to careful sterilization by autoclaving (121 ˚C for 20 minutes). Concentrations of oil and aqueous phase surfactant *i.e.* Span 80 and Tween-80, respectively were determined to maintain the final hydro and lipophilic balance of the FMD oil based vaccine at 7.0. One portion of inactivated FMD antigen was mixed with four portions of autoclaved plant oils comprising surfactants that resulted in water–in–oil (W/O) mixture as stated by (Stone, 1988).

Sterility and stability tests of vaccines

Sterility assays of all prepared and commercial vaccines were performed by carrying out inoculation of these vaccines on different microbiological media, *i.e.* Nutrient agar, Robertson's cooked meat broth, Blood agar, Sabouraud agar, MacConkey's agar, and PPLO agar at 37 ˚C for a period of 1 to 2 days (Mahboob et al., 1996). Physical stability factors of the shot like viscosity, color, vaccine consistency and blend type were also observed as designated by Stone (1988).

Safety test

Each vaccine was injected at tongue (intradermal route) of each goat at the rate of 100 µl. Afterwards, 2 ml of vaccine was injected on the neck of previously inoculated animals at day 4. The goats were kept under rigorous observations for following 6 days in order to observe any clinical signs of FMD (Cloette et al., 2008). This study was approved by the Institutional Ethical Committee of University of Agriculture, Faisalabad for care and use of animals during experimentation.

Experimental model

The entire experiment was conducted on 30 female goats of 1 to 2 years of age maintained in field conditions. Only those animals were chosen for experiment which were not previously inoculated with vaccine of FMD and were found to be sero-negative by 3 ABC-ELISA (which indicates no previous viral infection). These animals were separated into five groups, so each group had six goats. These goats were immunized with the first shot on day 1 and then subsequently, 21 days afterward the first dose by intramuscular (IM) route, with FMD vaccines comprising various adjuvants as follows (Table 1).

Collection of sera samples

Samples of blood deprived of anticoagulants were collected from the goats by 3 ml sterile disposable syringe from day 0 to day 28 weekly and then afterwards fortnightly till day 70 post vaccination. After collection blood samples were instantly transferred to sterile test tubes, placed in inclined position and stored in refrigerator for whole night. Next day serum was collected and stored at -20 ˚C for further use.

Table 1: Experimental Model for Vaccination of Goats

Experimental Group	Total number of goats	Vaccine adjuvant used	Dose of Vaccine (ml)	Vaccine administration route
A	6	Aluminum hydroxide and saponin (AS)	2	IM
B	6	Peanut oil	2	IM
C	6	Sunflower oil	2	IM
D	6	Olive oil	2	IM
E	6	Normal saline	2	IM

Group A: control positive (given a commercial FMD vaccine having AS adjuvant); Group E: Control negative (given no vaccine but normal saline).

Indirect Haemagglutination assay (IHA)

Prior to IHA, serum samples were exposed to temperature of 56ºC for 45 minutes in water bath, for deactivating the complement and nonspecific inhibitors. The protocol followed for IHA was as described by Xiao et al. (2007).

Physical Parameters of Experimental Animals

Physical parameters e.g. temperature, inflammation at the place of vaccine injection and death of animals were monitored and documented in all experimental groups for one week following initial and subsequent booster shot of vaccine.

Statistical Analysis

Geometric Mean Titer (GMT) was calculated by using the serum titers for each experimental group. Cumulative Mean Titer (CMT) was calculated by using the serum titer of each experimental group in all the weeks. Statistical differences among GMTs and CMTs of different groups within each experiment were estimated using the analysis of variance (ANOVA) and means were compared by applying Duncan's multiple range (DMR) test (Duncan, 1955; Steel and Torrie, 1980).

RESULTS

Sterility test results showed that all the vaccine preparations were germ-free and no growth was observed when inoculated on different bacterial media. Thus all of the preparations were harmless, germ-free and stable. Different physical parameters (vaccine viscosity, color and emulsion type) of oil based FMD vaccines were recorded and results are tabulated and presented in Table 2. Regarding safety of the shots, none of the vaccinated animals showed any sign of infection or tissue inflammation.

Humoral immune response of goats against FMD vaccination

At the day of first shot of vaccination, Geometric mean titers (GMTs) of various groups extended from 2 to 8. The difference in the GMTs was found to be non-significant ($P>0.05$) on the day of vaccination. At day 7, 14 and 21 post vaccination, a sharp increase in the antibody titers was found, GMTs of group A was found to be the highest followed by group B and C even if this change was not statistically important ($P>0.05$), but, group A and B GMTs were significantly different ($P<0.05$) from group D. GMTs of all vaccinated groups except group D were also meaningfully greater ($P<0.05$) than non-vaccinated E group at day 7, 14 and 21.However, following the booster dose of vaccine on day 21, it was found that the increase in the titers of vegetable oil adjuvanted FMD vaccines were seemed to be much higher than Aluminum hydroxide and saponin (AS) adjuvanted FMD commercial vaccine. So titers of oil adjuvanted vaccines were found to be comparable to the AS adjuvanted FMD vaccines at day 56 and 70 following vaccination. Furthermore, after booster dose, statistically, no significant change was seen ($P>0.05$) among A, B, C and D groups, however, Group A, B, C and D varied considerably ($P<0.05$) when matched to the negative control (group E) (Table 3).

Possible adverse reactions (systemic and local) of different FMD vaccines were also monitored for a period of 7 days after first and booster dosage of vaccination. Body temperature of different groups were noted and no important change ($P>0.05$) in physical temperature was found following initial and booster dose of immunization. The body temperature (°F) of A, B, C, D and E groups ranged from 101.6-103.6, 101.8-103.6, 102.0-103.6, 101.6-103.8 and 101.8-103.8, respectively following priming dose of immunization. Mean body temperature was 102.4, 102.6, 102.5, 102.8 and 102.7 for group A, B, C, D and E respectively. While following booster dose, group A, B, C, D and E had a body temperature ranging from 101.8 to 103.6, 101.6 to 103.4, 101.2 to 103.0, 101.6 to 103.2 and 101.6 to 103.8 respectively (observed for a period of 7 days). Mean body temperature was 102.6, 102.5, 102.4, 102.5 and 102.3 for group A, B, C, D and E respectively. Statistically, difference among means of body temperature of different groups was found insignificant ($P>0.05$) following priming and booster dose of immunization.

Mortality rate of animals were also recorded following vaccination (priming and booster shot). No mortality was recorded in any of the group, so on the basis of these findings all of the vaccines were “safe”.

Observations regarding swelling at injection site were also taken. No animal showed any sign of pain or localized swelling after priming dose of immunization. However, following booster dose of immunization, 5/6, 4/6, 5/6, 0/6 and 0/6 animals of group A, B, C, D and E showed localized inflammation at the site of injection.

Table II: Different physical parameters of vegetable oil based FMD vaccines

Oil used as adjuvant	Color of oil based vaccine	Vaccine viscosity	Type of Emulsion	Stability test (at 4)
Peanut oil	White creamy (light)	5.0 seconds	WO	Five months
Sunflower oil	White creamy(Dark)	6.0 seconds	WO	Five months
Olive oil	White creamy(Light)	4.5 seconds	WO	Five months

Table 3: GMTs of anti FMD antibodies in goats on post-inoculation of different oil based FMD vaccines

Days Post Vaccination	Group. A	Group. B	Group. C	Group. D	Group. E
0	3.56[a]	3.17[a]	3.17[a]	2.24[a]	2.24[a]
7	8.98[a]	7.13[ab]	5.66[bc]	3.18[c]	2.52[d]
14	20.16[a]	17.96[a]	14.25[ab]	8.98[b]	3.17[c]
21	35.92[a]	40.32[a]	35.92[a]	22.63[a]	3.56[b]
28	71.84[a]	64[a]	57.02[a]	50.80[a]	4.49[b]
42	90.51[a]	90.51[a]	71.84[a]	64[a]	5.65[b]
56	101.59[a]	114.04[a]	101.59[a]	90.51[a]	5.65[b]
70	101.59[a]	128[a]	114.04[a]	101.59[a]	6.35[b]
CMT	46.00[a]	46.00[a]	39.00[a]	32.00[a]	3.94[b]

Means indicating the same letters are not different at ($P<0.05$) in different groups (rows) at different time intervals (columns).

DISCUSSION

The results of present study showed that various vegetable oils have potential to be used as vaccine adjuvants, an addition to the traditionally used adjuvants. Peanut oil, sunflower oil and olive oil enhanced the humoral responses against FMD following priming and booster dose, although after booster dose, rate of increase in antibody titer was quicker when matched with the AS adjuvanted commercial FMD shot. These results recommend that these oils have ability to up regulate the Th2 reaction.

Although vegetable oil adjuvanted shots resulted in an enhanced humoral immune response against FMD but still exact mechanism cannot be explained on the basis of present study. Two different possibilities may exist: First, peanut oil, sunflower oil and olive oil may have ability to improve the humoral immune reaction by the depot effect which they create locally. These oils allow the slow and sustained release of antigen for a longer period of time, which results in prolong motivation of immune system and thus resulted in higher antibody titers. For example, Sartor et al. (2011) evaluated the adjuvant activity of Rice Oil (RO) w.r.t. immune response induced by ovalbumin. It was revealed that RO emulsified ovalbumin resulted in prolonged immunization (as long as 6 weeks) as compared to ovalbumin emulsified in incomplete Freund's adjuvant (IFA). Second, these oils also contain a variety of immunomodulatory compounds e.g. vitamin E, oleic acid and linoleic acid as observed by Calder (1998). Peanut oil contains higher amount of oleic acid (62%), so it resulted in significant enhancement of humoral responses against disease.

These results are in consonance with the Ezeifeka et al. (2008) in which ground nut oil seen to be the best adjuvant of Newcastle disease (ND) followed by soybean, olive and palm oil. These finding are also in contract with Wanasawaeng et al. (2009) in which peanut oil significantly enhanced the humoral response against ND and also resulted in some local tissue reactions following intramuscular administration of vaccine. The results of present study also partially provision the results of Freitas et al. (2013) who argued that the cottonseed, peanut and rice oil are capable of enhancing immune response against ovalbumin. No adverse reactions were observed in the study of Freitas et al. (2013) which is contrary to the present study, as in the present experiment all of the vaccines except olive oil adjuvanted FMD vaccine induced local reactions after booster dosage of immunization.

The current study therefore suggests the use of variety of locally available vegetable oils as adjuvants in various vaccine preparations. Using vegetable oils as adjuvants will result in better protection and immune response as well as cost effective production of vaccines using cheaper but effective adjuvants.

Authors' contributions

BM, MA conceived the idea, considered the experiment and carved the document. BM and SA performed the experiments and statistical analysis. SA, MA, UHK, AF and AW contributed in the project of the study and aided in writing the document. All authors read and accepted the final copy.

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