

Short Communication

ANTIBODY RESPONSE OF GOATS TO GEL BASED COMBINED VACCINE AGAINST PESTE DES PETITS RUMINANTS, CONTAGIOUS CAPRINE PLEUROPNEUMONIA AND FOOT AND MOUTH DISEASE

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ABSTRACT

Present study was designed to prepare and evaluate gel based combined vaccine against Peste des Petits Ruminants (PPR), Contagious Caprine Pleuropneumonia (CCPP), Foot and Mouth Disease “O serotype” (FMDO) to mitigate the cost and frequency of the vaccination. Gel based vaccines contain either single immunogen (monovalent) of PPR, CCPP or FMDO, two (bivalent) PPR+FMDO, PPR+CCPP, FMDO+CCPP or three (trivalent) PPR+CCPP+FMDO. Each dose (0.5 ml) of either of the vaccines contained 10⁵ tissue culture infective dose (TCID₅₀) units of FMDO virus, 10⁷ TCID₅₀ units of PPR virus and 0.15 mg of *Mycoplasma mycoides capri*. Each of the vaccine was injected subcutaneously to each of the goats (n=4). Antibody response of goats to FMDO, PPR and CCPP was determined by complement fixation test (CFT), virus neutralization test (VNT) and enzyme linked immunosorbant assay (ELISA), respectively at 0, 60, 120 and 180 days post-priming. Antibody response of the goats to either of the immunogen was not significantly different irrespective to the form of vaccine (monovalent, bivalent or multivalent vaccine) (P>0.05). It indicated that either of the immunogen did not interfere the immunogenesis process of other immunogens in the same vaccine.

Key words: Peste des Petits Ruminants, Foot and Mouth Disease, Contagious Caprine Pleuropneumonia, Combined vaccine

INTRODUCTION

Goat being recognized as poor man’s cow is an integral part of the livestock which shares 58.33% to the value addition in agriculture sector and about 11.4% of GDP of Pakistan. Almost 30-35 million rural population is involved in livestock production. Most of the families have 2-3 cattle/buffalo and 5-6 sheep/goats. People residing in rural areas are usually of low socioeconomic status and their main source of income is domestic animals. In livestock sector, goat population shares 72.2 million and goats are helpful in fulfilling the increasing demand of milk, meat, skin and leather (Annonymous, 2016-2017).

Raising small ruminants is a profitable business but its mass production is hampered by low genetic potential, malnutrition, reproductive disorders, diseases (primarily viral and bacterial), poor husbandry practices and endoparasites and ectoparasites. Other factors responsible for low profit are lack of knowledge, unorganized farming and costly vaccines. Out of these factors, microbial diseases are the prime hindrance in the profitability of goat farming. Goat farmers are usually poor so are reluctant to manage costly vaccines. Goat diseases are a biggest constraint in economic growth, poverty reduction and food security (Depa *et al.*, 2012). Contagious caprine pleuropneumonia (CCPP) is a huge threat to goats and it is distributed widely in Pakistan

(Sadique *et al.*, 2012; Tarekegn *et al.*, 2012). Foot and Mouth Disease O serotype (FMDO) is a most significant viral disease which is highly contagious, affecting multi species primarily cloven-footed animals including goats (Gorsi *et al.*, 2011; Depa *et al.*, 2012). Peste des Petits Ruminants (PPR) result in 80-90 % morbidity and 50-80% mortality (Khan *et al.*, 2007).

Diseases in animals are controlled through mass scale vaccination all over the world. Currently available vaccines are costly and induce immunity for short time and hence are repeated after short intervals (Parida, 2009). Costly vaccines, short-lived immunity, increased vaccination frequency, etc., are the limiting factors in application of any mass vaccination programme. The present study was therefore designed to prepare and evaluate gel based combined vaccine against contagious caprine pleuropneumonia (CCPP), Peste des Petits Ruminants (PPR) and Foot and Mouth Disease “O” serotype (FMDO) in goats.

MATERIALS AND METHODS

Sources of cell lines and viruses: Vaccine strain of PPR virus, FMDO virus and Vero and Baby Hamster Kidney (BHK-21) cells were procured from Department of Microbiology, University of Veterinary and Animal Sciences, Lahore. CCPP vaccine in lyophilized form was

obtained from Veterinary Research Institute (VRI) Lahore.

Propagation of viruses: The PPR and FMDO viruses were propagated on Vero and BHK-21 cells, respectively (OIE, 2008). Tissue culture infective dose 50 (TCID₅₀) was determined according to Reed and Muench method (Swayne *et al.*, 1998).

Inactivation of foot and mouth disease virus: The FMDO virus suspension of known TCID₅₀ was inactivated by binary ethylene imine (BEI) solution. The mixture was incubated at 37°C for 24 hour. The mixture was agitated, BEI was added again and incubated at the same temperature for the same time to ensure thorough inactivation. The residual BEI was neutralized by adding 2% sodium thiosulfate and incubating the mixture for 1 hour (Bahnmann 1990).

Formulation of vaccines: The gel based monovalent, bivalent and trivalent (combined) PPR, FMDO and CCPV vaccines were prepared (OIE, 2008). One dose of each of the vaccines was 0.5ml. The vaccines were prepared in following formulations. *Mycoplasma mycoides* suspension (15mg/100ml=0.1ml), 0.2ml of PPR virus with 10⁴ units of TCID₅₀ in monovalent, 0.02ml of PPR virus with 10⁵ units of TCID₅₀ in combined vaccines, 0.2ml of FMD O virus with 10⁶ units of TCID₅₀ in monovalent and 0.02ml of FMD O virus with 10⁷ units of TCID₅₀, in combined vaccine Aluminium hydroxide gel (4%) and Thiomersal Sodium (0.05%) were added in each of the vaccines as an adjuvant and preservative, respectively.

Vaccine tests (safety and sterility tests): Sterility tests were conducted to evaluate the vaccines for any bacterial or fungal contamination. The vaccines were tested for any untoward local or systemic reactions by safety test (OIE, 2008).

Vaccine inoculation: A total of 32 goats (Beetal breed) of age ranging from 1-3 years were selected from Karakol Sheep Research Farm, Maslakh, Quetta, Baluchistan. The goats were divided into eight groups (A, B, C, D, E, F, G and H) each having four animals. Each of the vaccines such as PPR, CCPV, FMDO, PPR+CCPV, FMDO+CCPV, FMDO+PPR and FMDO+PPR+CCPV were inoculated subcutaneously in each goat of group A, B, C, D, E, F and G, respectively. However, each animal of group H was kept as negative control.

Sample collection: Blood samples from each of the goats were collected on 0, 2, 4 and 6 months post vaccination. Serum from each of the blood samples was separated and stored in properly labeled vials at -40°C till further required for monitoring of the antigen specific antibodies.

Monitoring of antibody response: Antibodies titer against PPR virus, FMD O virus and *Mycoplasma*

mycoides (var capri) were measured through virus neutralization test (Swayne *et al.*, 1998), complement fixation test (CFT) and enzyme linked immunosorbent assay (OIE, 2008), respectively.

RESULTS AND DISCUSSION

Combined or multivalent vaccines are those having immunogens of ≥ 2 microbial pathogens. In the present study, combined gel based vaccines contained immunogens of PPR virus, *Mycoplasma capri* and FMD virus serotype O. The use of multivalent viral vaccines in goats is innovative. A number of multivalent vaccines against goat, bovine, canine and poultry microbial diseases are used to mitigate the cost and distress related with multiple injections (Gomes *et al.*, 2016; Fakri *et al.*, 2015; OIE and FAO, 2015; Chaudhary *et al.*, 2009).

Single dose of combined vaccine induced protective level of antibody response. Antibody response of the host depends upon amount of the immunogen in the vaccine (Akram *et al.*, 2012; Anees *et al.*, 2013; Sarwar *et al.*, 2012). Combined vaccine without any adjuvant is absorbed from the host inoculation site and is excreted out after degradation from the body. Such vaccine may fail to induce detectable level of antibody response. Adjuvants are therefore added in the vaccines to potentiate the retention time of the immunogen. Antibody response of the host is directly proportional to the retention time of the immunogen. Combined vaccine containing gel induced detectable level of antibody response. Aluminium hydroxide gel adsorb the immunogen and do not allow its absorption from the inoculation site hence enhance its retention time (Ghimire, 2015; Muhammad *et al.*, 2013; Lindblad 2004). In addition to gel, alum and oil are used as adjuvant in animal vaccines. Oil encapsulates the immunogen, make them insoluble and hence increase the retention time of the immunogens (Anajafi and Mallik, 2015; Sarwar *et al.*, 2012).

Monovalent gel based FMD serotype O (FMDO) vaccine induced gradual increase of anti-FMDO-CFT antibody response up to 120 days in goats and then started declining up to 180 days that was still protective titre (Table 1). Monovalent gel based PPR vaccine induced increasing trend of anti-PPR-VNT antibody response till end of the experiment (Table 2). PPR vaccine containing either field isolate (China/Tib/07) or vaccine strain (Nigeria 75/1) induced ≥ 8 units of anti-PPR-VNT antibody (protective level) that persisted up to eight months post-vaccination (Liu *et al.*, 2012). Monovalent gel based GCCPV vaccine induced 64 percent of anti-CCPV-ELISA antibody titre on 60 days post-priming and gradually decreased thereafter up to 180 days post-priming (Table 3). Live vaccine induces higher antibody response in goats as compared to killed vaccine (Schieck *et al.*, 2016; Tarekegn *et al.*, 2012).

Bivalent FMDO+PPR vaccine induced anti-FMDO-CFT and anti-PPR-VNT antibody response in the goats that was not significantly different from that of the goats vaccinated with either of monovalent vaccine (Table 1 and 2). Bivalent FMDO+CCPP vaccine induced anti-FMDO-CFT and anti-CCPP-ELISA antibody response of the goats that was not significantly different from that of the goats vaccinated with either of monovalent vaccine (Table 2 and 3). Bivalent PPR+CCPP vaccine induced anti-PPR-VNT and anti-CCPP-ELISA antibody response in the goats that was not significantly different from that of the goats vaccinated with either of monovalent vaccine (Table 2 and 3). Bivalent vaccine containing attenuated PPR virus and sheep pox virus (SPV) induces the same anti-PPR-VNT antibody response in sheep as that of monovalent PPR vaccine (Chaudhary *et al.*, 2009). In bivalent vaccine one immunogen do not interfere the response of other immunogen so bivalent vaccines are effective way to induce immunoprophylaxis simultaneously against both of the diseases (Shakoor *et al.*, 2013).

Combined FMDO+PPR+CCPP vaccine induced anti-FMDO-CFT, anti-PPR-VNT and anti-CCPP-ELISA antibody response of goats that was not significantly different from that of the goats vaccinated with either monovalent or bivalent vaccine (Table 1, 2 and 3). In host vaccinated with multivalent vaccine, each immunogen is processed and recognized by its specific immunocytes (B cells and T_h cells) for induction of immunoprophylaxis against the respective diseases. In the present study no immunogen interfered immunogenesis process of other immunogens. In vaccinated goats anti-PPR-VNT

antibodies ≥ 8 is a protective titre. Gel based inactivated quadrivalent FMD vaccine containing serotype O, A, C and Asia-I induces protective titre up to 120 days whereas oil based vaccine induces protective titer up to 270 days post-vaccination (Madhanmohan *et al.*, 2008). A multivalent vaccine having 250 HU of *Clostridium chauvoei*, 0.2×10^7 TCID₅₀ units of FMD virus serotypes A, O and Asia-I and 2 mg of *Pasteurella multocida* induces a protective immune response in buffalo calves (Farooq, 2013).

It is concluded that in multivalent vaccine for goat, each of the immunogens does not interfere immunogenesis pathway of the others and hence induces effective immuno-prophylaxis simultaneously against all the diseases.

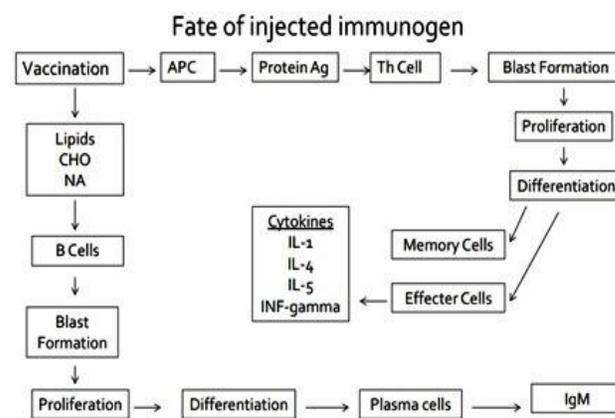


Table 1. Antibody response of goats to foot and mouth disease (FMD type O) virus vaccines.

Vaccine Type	Anti-FMD type O-CFT Antibodies Titer at Days (GMT)				CGMT Log ₂ (mean±SD)
	0	60	120	180	
FMDO	0	4.75	5.25	4.25	4.73 ^a ±0.41
FMDO+PPR	0	4.75	5.75	5.0	5.22 ^a ±0.36
FMDO+CCPP	0	4.95	4.95	5.89	5.24 ^a ±0.44
FMDO+PPR+CCPP	0	5.18	5.14	5.14	5.15 ^a ±0.02

Note: Figures in last column showing similar superscript are not significantly different (p>0.05), PPR: Peste des petits ruminants, CCPP: Contagious caprine pleuropneumonia.

Table 2. Antibody response of goats to peste des petits ruminants (PPR) virus vaccines.

Vaccine Type	Anti-PPRV-VNT Titer at Days (GMT)				CGMT Log ₂ (mean±SD)
	0	60	120	180	
PPR	0	4.75	5.5	5.75	5.32 ^a ±0.43
PPR+FMDO	0	5.0	6.25	6.0	5.72 ^a ±0.54
PPR+CCPP	0	4.75	6.0	6.75	5.83 ^a ±0.82
PPR+FMDO+CCPP	0	5.25	5.75	6.25	5.75 ^a ±0.41

Note: Figures in last column showing similar superscript are not significantly different (p>0.05) FMDO: Foot and mouth disease virus-Serotype O, CCPP: Contagious caprine pleuropneumonia

Table 3. Antibody response of goats to contagious caprine pleuropneumonia (CCPP) vaccines.

Vaccine Type	Anti CCPP-ELISA Titre at Days Post-priming (Mean Titres) (O.D-Optical Density Values)				Mean ± SD
	0	60	120	180	
CCPP	0	0.58	0.63	0.68	0.63 ^a ±0.05
CCPP+FMD	0	0.57	0.62	0.67	0.62 ^a ±0.05
PPR+CCPP	0	0.58	0.61	0.63	0.64 ^a ±0.07
PPR+FMD+CCPP	0	0.59	0.62	0.64	0.64 ^a ±0.06

Note: Figures in last column showing similar superscript are not significantly different ($p>0.05$)

PPR: Peste des petits ruminants, FMD: Foot and mouth disease virus

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