Antibody Response of Goats to Bivalent Peste des Petits Ruminants Virus and Goat Pox Virus Vaccine

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Abstract: Peste des Petits Ruminants (PPR) and Goat pox (GP) are the most acute viral disorders of sheep, goats, deer and other similar animals and are controlled through annual mass vaccination programs. The objective of the study was to prepare bivalent Peste des Petits Ruminants Virus (PPRV) and Goat Pox Virus (GPV) vaccine and evaluate humoral immune response in goats against both immunogens. All animals included in the study were dewormed and screened for any existing diseases before vaccination trials. Serum antibody response of the goats was monitored on zero, 21, 42, and 63 days post-vaccination through complement fixation test (CFT). There was a non-significant difference between the antibody response of goats to Freeze-dried monovalent and freeze-dried bivalent vaccines ($p>0.05$). Antibody response of goats to either of the immunogen (PPRV and GPV) was directly proportional to the amount of the immunogen in the bivalent Gel based-PPRV+GPV vaccine. Bivalent vaccines with similar titers of the viruses ($10^{3.5}$ units TCID50) either in freeze-dried form, adjuvanted with montanide 50 oil or aluminum hydroxide gel, triggered non-significantly different ($p>0.05$) antibody response in goats. It was concluded from the study that bivalent vaccine of PPRV+GPV produced antibodies in goats and the vaccine is required to have at least $10^{3.5}$ TCID50 units of each of the viruses. Finally, an effective vaccine can be prepared in freeze-dried stabilizer, oil or gel-based adjuvants.

Key words: Bivalent vaccine, Complement fixation test, Goat Pox virus, Peste des Petits Ruminants virus

INTRODUCTION

Peste des Petits Ruminants (PPR) is one of the most acute viral diseases of sheep, goats, deer and other small ruminants. The disease is caused by Morbillivirus of Paramyxoviridae and characterized by oculo-nasal discharge, stomatitis, diarrhea, high temperature, and pneumonia with foul breath. It is transmitted by aerosols to other susceptible animals in the vicinity of the infected animal (9). PPR is endemic in most parts of the world and each year causes severe economic losses in the form of animal mortalities and morbidities. It causes 100% morbidity and fatality in serious outbreaks (13). Similarly, Pox is an acute and febrile viral disease of sheep, goats, and cattle caused by Capri poxvirus of Poxviridae. It is characterized by high temperature, and different stages of pox lesion development such as vesicles, scars, pustules, erythema, and papules, all over the body. The nodular lesions are visible on the dental pad and the lips. The disease causes 70-90% morbidity and 5-10% mortality in prevalent areas. (8,17).

Both FAO and OIE are collaborating to eradicate PPR through mass vaccination by its global eradication program (GCSE) launched in 2015 (13). Vaccination is said to be the most efficient and cost-effective way for control, prevention and eradication of diseases. In most vaccination programs the cost of vaccine storage, cold chain maintenance, transport, and vaccination staff surpasses the original cost of vaccine production. To reduce these expenses, many

individual vaccines can be combined safely in bivalent, trivalent and multivalent forms (19).

Throughout the globe, many combined commercialized vaccines are in practice not only against human diseases (diphtheria/tetanus/pertussis or mumps/measles/rubella) but also targeting the diseases of livestock, poultry and other domestic animals. PPR vaccine is produced and administered with many other vaccines in combined form such as; peste des petits ruminants+goat pox virus, peste des petits ruminants + contagious caprine pleuro-pneumonia, peste des petits ruminants + foot and mouth disease, peste des petits ruminants+ rift valley fever and peste des petits ruminants+ trypanosomosis. In addition, vaccines against pasteurellosis, brucellosis, anthrax, and enterotoxemia are also produced in a combined form with the PPR vaccine (21). The present study was planned to prepare a combined PPR virus and goat pox virus vaccine and monitor the antibody response of goats to the vaccines. Efforts were made to investigate the effect of adjuvants (Montanide 50 oil and aluminum hydroxide gel) as well as the amount of immunogen on the antibody titers of goats.

MATERIALS AND METHODS

***Propagation and harvesting of the viruses*:** Cell culture medium Dulbecco`s Modified Eagle Medium (DMEM) was prepared and maintained as per Freshney's protocol. Vero cell line obtained from Veterinary Research Institute (VRI) Lahore, Pakistan was revived and propagated for virus growth. Cell lines were infected with PPR (Nigeria 75/1) and Goat Pox (Gorgan 55) vaccine strains viruses, obtained from VRI Lahore, and the viruses was harvested for vaccine preparation. TCID50 for both viruses was calculated by the Spearman-Karber method.

Preparation of various vaccines:Freeze-dried vaccine of PPRV was prepared in Weybridge medium (Lactalbumin hydrolysate, sucrose, sodium glutamate) and that of GPV in LPS (Lactose, Peptone, Sucrose). The virus and stabilizer were mixed together in equal volumes and freeze-drying was carried out with Heto Power PL6000 Freeze Dryer. Combined PPRV+GPV vaccine was prepared by adding and freeze drying 1ml PPRV, 1ml GPV and 2 ml stabilizer (5).

Oil-based combined vaccine was prepared by mixing 1ml PPRV, 1ml GPV, 48ml PBS, 50ml montanide oil ISA 50 and preservative thiomersal sodium in the final concentration of 0.01% (20).

Three types of gel-based combined PPRV+GPV vaccines were prepared each having $10^{2.5}$, $10^{3.5}$, and $10^{4.5}$ biological titers. The volume required for each biological titer of virus harvests was measured and added to PBS with 2%gel in a 50:50 ratio. Preservative thiomersal sodium was added in the final concentration of 0.01% (Table # 1).

***Animals selection and their grouping*:**Total of 42 goats reared at Maslakh Small Ruminants Research Farm Quetta Baluchistan were selected for vaccine trials. Seven groups of goats were arranged with each group containing six goats for inoculation of each type of vaccine. All animals were healthy and dewormed with Albendazole before inoculation of the vaccine. Vaccine inoculation was as; goats in group A with freeze-dried PPR, goats of group B with freeze-dried GPV, group C with bivalent PPR+GPV freeze-dried, group D with bivalent PPR+GPV oil-based, group E with bivalent PPR+GPV gel based, group F with bivalent PPR+GPV gel based $10^{4.5}$ TCID50, group G with bivalent PPR+GPV gel based $10^{2.5}$ TCID50.

Note: Goats in groups A, B, C, D, and E were inoculated with a standard amount of each of the immunogens ($10^{3.5}$ TCID50).

***Serum sampling and CFT*:**3ml Blood sample was collected from the jugular vein of the animals in a plain vacutainer on day zero (0) for confirmation of no antibodies against PPRV or goat pox followed by day 21st, 42nd, and 63rd of inoculation. Serum in the supernatant was taken with a pipette and shifted to a 1.5ml capacity Eppendorf tube and stored at -40C till further processing. The antibodies in each of these samples were determined by complement fixation test (CFT), using multiple channels micro plastic plates 96 wells of titration (Marchand and Packer, 1983). Amboceptorwas raised in rabbits and Guinea pig's serum complement was used as an indicator for this test.

STATISTICAL ANALYSIS: The antibody titers of goats were measured at days 21, 42, and 63 post vaccinations by calculating GMT log2 values of well numbers. One-way ANOVA test was applied to the data for comparing geometric mean titers of the vaccine results in SPSS version 20.

RESULTS

Comparison of monovalent PPRV, monovalent GPV,and bivalent PPRV+GPV vaccines:Geometric mean antibody titer of goats in group A against monovalent freeze-dried PPRV vaccine was $\log_2$ 3. Likewise, the GM antibody titer of animals in group B towards monovalent freeze-dried GPV vaccine was also $\log_2$ 3. The antibody titers in these monovalent vaccines were compared with the GMT of bivalent freeze-driedvaccines. The serum of animals in group C (inoculated with bivalent FD PPRV+GPV vaccine) was tested for both types of antibodies separately. GM antibody titer against PPRV and GPV in bivalent FD vaccine was $\log_2$ 2.66 and $\log_2$ 2.5 respectively. The data was subjected to one-way ANOVA for statistical analysis and the outcomes showed that the antibody response of goats to PPRV and GPV in the monovalent vaccine was not significantly different from that of the PPRV+GPV in bivalent form (P>0.05) (Table # 2).The GM antibody titers mentioned above were monitored 63 days post-vaccination.

Effect of different adjuvants on antibody response of goats to bivalent PPR+GPV vaccine: Geometric mean titer of the goats against PPRV and GPV in bivalent FD PPPRV+GPV vaccine was $\log_2$ 2.66 and $\log_2$ 2.5 respectively. Similarly, the Geometric mean titer of the goats against PPRV and GPV towards oil (montanide oil ISA-50) based bivalent PPRV+GPV vaccine was $\log_2$ 3 and $\log_2$ 3 respectively. Finally, the CFT Geometric mean titer of the goats against PPRV and GPV towards gel (aluminum hydroxide gel) based bivalent PPRV+GPV vaccine was $\log_2$ 3 and $\log_2$ 3 respectively. The data was subjected to one-way ANOVA for statistical analysis and the outcomes showed that antibody response of goats to PPRV and GPV in bivalent FD PPRV+GPV, oil-based PPRV+GPV and gel based PPRV+GPV vaccines was not significantly different from each other (P>0.05) (Table # 3).The GM antibody titers mentioned above were monitored 63 days post vaccination.

Effect of amount of immunogen in gel based combined PPRV+GPV vaccines on antibody response of goats: Three aluminum hydroxide gel based bivalent PPRV+GPV vaccines containing $10^{2.5}$,$10^{3.5}$ and $10^{4.5}$ TCID units/ml were inoculated in three various groups of animals. The geometric mean antibody response of goats towards PPRV and GPV in$10^{2.5}$ bivalent PPRV+GPV vaccine was $\log_2$ 0.16 and $\log_2$ 0.16 respectively. Geometric mean antibody response of goats towards PPRV and GPV in$10^{3.5}$ bivalent PPRV+GPV vaccine was $\log_2$ 3 and $\log_2$ 3 respectively. In contrast, the geometric mean antibody response of goats towards PPRV and GPV in$10^{4.5}$ bivalent PPRV+GPV vaccine was $\log_2$ 4 and $\log_2$ 4 respectively. The data was subjected to one way ANOVA for statistical analysis and the outcomes showed that antibody response of goats to PPRV and GPV in bivalent gel-based PPRV+GPV vaccines containing $10^{2.5}$,$10^{3.5}$ and $10^{4.5}$ TCID units/ were significantly different from each other (P<0.05) (Table # 4).The GM antibody titers mentioned above were monitored 63 days post-vaccination.

DISCUSSION AND CONCLUSION

Peste des Petits Ruminants (PPR) and Goat pox (GP) are the most acute viral disorders of sheep, goats, deer, and other similar animals and are controlled through annual mass vaccination programs (9). An attenuated strain of PPR Nigeria 75/1 is used to vaccinate the animals in Pakistan and other PPR endemic countries (1). Vaccination is the most efficient and cost-effective way to control, prevention, and eradication of the diseases. In most vaccination programs the cost of vaccine storage, cold chain maintenance, transport, and vaccination staff surpasses the original cost of vaccine production. To reduce these expenses, many individual vaccines can be combined safely in bivalent, trivalent, and multivalent forms (19). The purpose of the current study was to prepare a combined PPR virus and goat pox virus vaccine and monitor the antibody response of goats to the vaccines. Furthermore, investigating the effect of adjuvants (Montanide 50 oil and aluminum hydroxide gel) and the amount of immunogens on the antibody titers of goats were also objectives of the study.

Geometric mean CFT antibody titer of goats against monovalent freeze dried PPRV vaccine was $\log_2$3. Likewise, the GM antibody titer against monovalent freeze dried GPV vaccine was also $\log_2$3. The antibody titer of goats inoculated with bivalent FD PPRV+GPV vaccine was $\log_2$ 2.66 and $\log_2$ 2.5 respectively. Statistical analysis showed that antibody response of goats to PPRV and GPV in monovalent vaccine was not significantly different from that of the PPRV+GPV in bivalent form (P>0.05). Similar

results were noted by Fakri et al. (2015) stating that bivalent PPR + Sheep Goat Pox (SGP) vaccine induced a protective antibody response and this response was not significantly different from monovalent PPR and SGP vaccines. (15) also found that the bivalent Peste des petits ruminants virus (PPRV) and goat poxvirus (GPV) vaccine was safe and the vaccine produced a protective immune response in goats. Moreover, further studies were conducted by (11,12) with similar outcomes suggesting that the combined PPR+GPV vaccine was equally potent and safe to use.

In addition to combined PPRV+GPV vaccines, many researchers have found out that other bivalent and multivalent viral vaccines, like; Rift Valley fever (RVF), bluetongue (BT); Canine Distemper Virus, Canine Adenovirus and Canine Parvovirus; and Bovine Viral Diarrhea virus type 1, Bovine Viral Diarrhea virus type 2, Infectious Bovine Rhinotracheitis virus, Bovine Parainfluenza-3 virus, Bovine Respiratory Syncytial virus; are safe, equally potent and protective (10, 26, 27).

The effect of Freeze-dried stabilizer, oil (montanide ISA-50) and aluminum hydroxide gel in bivalent PPRV+GPV vaccine was also analyzed in the current study. The geometric mean antibody titer of the goats against bivalent FD PPPRV+GPV vaccine was $\log_2$ 2.66 and $\log_2$ 2.5 respectively. Similarly, the Geometric mean antibody titer of the goats against oil-based bivalent PPRV+GPV vaccine was $\log_2$ 3 and $\log_2$ 3 respectively. Finally, the CFT Geometric mean antibody titer of the goats against gel based bivalent PPRV+GPV vaccine was $\log_2$ 3 and $\log_2$ 3 respectively. On statistical analysis, the outcomes showed that the antibody response of goats to PPRV and GPV in bivalent FD PPRV+GPV, oil-based PPRV+GPV, and gel-based PPRV+GPV vaccines was not significantly different from each other ($P>0.05$). All the vaccines contained an equal amount of immunogen which was $10^{3.5}$ TCID50 units/ml. The GM antibody titers mentioned above were monitored 63 days post-vaccination. Many adjuvants like montanide oil, aluminum hydroxide gel, and saponins are commonly used in veterinary vaccines (6). In contrast to the current study (5) studied theoil-based PPRV vaccine induced better antibody response even after one year post-vaccination by using$10^{4.5}$ TCID units of the virus. This could be due to using a higher amount of PPRV immunogen in the oil-based vaccine. (3) also noted that oil-based FMDV vaccine induced significantly higher antibody titers in sheep compared to gel-based and nonadjuvanted FMDV vaccines. The reason for higher antibody titers against oil-basedvaccines could be due to monitoring the antibody titers for a longer duration (1 year) of time as due to slow absorption in the tissue the titer of oil-based vaccines increases slowly and persists for longer duration (23). Aluminum hydroxide gel retains antigen for a short period and antibody response to gel-based vaccines starts more rapidly than that of oil-basedvaccines, reaches peak level, and declines rapidly (24).

Aluminum hydroxide gel-based bivalent PPRV+GPV vaccines containing $10^{2.5}$,$10^{3.5}$ and $10^{4.5}$ TCID units/ml were inoculated in three various groups of animals. The geometric mean antibody response in$10^{2.5}$ bivalent PPRV+GPV vaccine was $\log_2$ 0.16 and $\log_2$ 0.16 respectively. The geometric mean antibody response in$10^{3.5}$ bivalent PPRV+GPV vaccine was $\log_2$ 3 and $\log_2$ 3 respectively. In contrast, the geometric mean antibody response in$10^{4.5}$ bivalent PPRV+GPV vaccine was $\log_2$ 4 and $\log_2$ 4 respectively. Statistical outcomes of the data showed that antibody response of goats in bivalent gel-based PPRV+GPV vaccines containing $10^{2.5}$,$10^{3.5}$ and $10^{4.5}$ TCID units/ were significantly different from each other ($P<0.05$). The GM antibody titers mentioned above were monitored 63 days post vaccination. The results of the current study were by the study of (5) stating that the PPRV vaccine containing 10^5 units of TCID50 induces maximum antibody titer in goats. (22) also noted that the immune response of goats to PPRV is dependent on the amount of the immunogen. Other animal species like buffalo and poultry birds also show immunogen concentration-dependent immune responses (16). Likewise, the increase in antibody titer of buffalo calves is directly proportional to the amount of virus titers in oil-based multivalent FMDV vaccine (25, 2, 3).

It was concluded from the study that the bivalent vaccine of PPRV+GPV produced antibodies in goats and the vaccine is required to have at least $10^{3.5}$ TCID50 units of each of the viruses. Effective vaccines can be prepared in freeze-driedstabilizers, oil, or gel-based adjuvants. This study recommends

that a bivalent (PPRV+GPV) vaccine be prepared. Equally, bivalent vaccines are more economical in storage, cold chain maintenance, transport, and vaccination staff costs. Finally, multivalent vaccines against as many as possible pathogens of the same animal species can be prepared.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authors contribution

Farooq M and Muhammad K planned and conceived the research. Farooq M and Latif MZ carried out the simulations and vaccine preparation. Farooq M,Khan MA, Mengal AN and Mengal S conducted the trials of the vaccine. Farooq M, Anjum AA, and Avais M contributed to the interpretation of the results.Farooq M took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Table No. 1. Seven type various vaccines with their level of immunogen and adjuvant type.

Animal groups	A	B	C	D	E	F	G
Vaccine inoculated (Adjuvant nature)	PPR (FD)	GP (FD)	PPR+GP (FD)	PPR+GP (Oil)	PPR+GP (Gel)	PPR+GP (Gel)	PPR+GP (Gel)
Amount of immunogen	$10^{3.5}$	$10^{3.5}$	$10^{3.5}$	$10^{3.5}$	$10^{3.5}$	$10^{4.5}$	$10^{2.5}$

Table No.2: Antibody response of goats to monovalent PPRV and monovalent GPV vaccines compared to bivalent PPR+GPV vaccine.

Vaccine type	Antibody response against	Antibody response of goats to monovalent (PPR virus) and bivalent (PPR+GP) vaccine on days post priming		
		21	42	63
FD-PPR virus vaccine	PPR virus	2,2,2,2,2,0(0.83)	4,4,4,4,4,4(2)	8,8,8,8,8,8(3)
FD-GP* virus vaccine	Goat Pox virus	2,2,2,2,2,2(1)	4,4,4,4,4,4(2)	8,8,8,8,8,8(3)
FD PPR+GP*	PPR virus	2,2,2,2,0,0(0.66)	4,4,4,4,2,2(1.66)	8,8,8,8,4,4(2.66)
	GP virus	2,2,2,0,0,0(0.5)	4,4,4,4,2,2(1.66)	8,8,8,4,4,4(2.5)

CFT (Complement Fixation Test) serum antibody titers of goats vaccinated with three types of different vaccines. FD PPRV= Freeze dried peste des petits ruminants virus, GPV*= goat Pox and bivalent PPRV+GPV. All the vaccines PPRV, GPV and PPRV+GPV contained equal amount of immunogen that was $10^{3.5}$ TCID50 units/ml.

Table No. 3. Effect of different adjuvants on antibody response of goats to bivalent PPR+GPV vaccine

Vaccine type	Antibody response against	Effect of different adjuvants on antibody response of goats to bivalent PPR+GPV vaccine on days post priming		
		21	42	63
FD**-PPR+GP	PPR virus	2,2,2,2,0,0(0.66)	4,4,4,4,2,2(1.66)	8,8,8,8,4,4(2.66)
	GP* virus	2,2,2,0,0,0(0.50)	4,4,4,4,2,2(1.66)	8,8,8,4,4,4(2.50)
Oil****-PPR+GP	PPR virus	2,2,2,0,0,0(0.50)	4,4,4,4,4,4(2)	8,8,8,8,8,8(3)
	GP virus	2,2,0,0,0,0(0.33)	4,4,4,4,4,4(2)	8,8,8,8,8,8(3)
Gel***-PPR+GP	PPR virus	4,4,2,2,2,2(1.33)	4,4,4,4,4,4(2)	8,8,8,8,8,8(3)
	GP virus	2,2,2,2,2,2(1)	4,4,4,4,4,4(2)	8,8,8,8,8,8(3)

CFT (Complement Fixation Test) serum antibody titers of goats vaccinated with three types of different vaccines. GP*= goat Pox, FD**=freeze dried, Gel***= aluminum hydroxide gel, Oil****=montanide oil ISA-50. All the vaccines contained equal amount of immunogen that was $10^{3.5}$ TCID50 units/ml.

Table No. 4. Effect of immunogen level on antibody response of goats to gel based bivalent PPR+GPV vaccine

Vaccine type	Amount of immunogen	Antibody response against	Effect of immunogen level on antibody response of goats to bivalent PPR+GPV vaccine on days post priming		
			21	42	63
Gel-PPR+GP	$10^{2.5}$ TCID	PPR virus	2,0,0,0,0,0(0.16)	2,2,2,2,0,0(0.66)	4,4,4,4,4,2(1.83)
	$10^{2.5}$TCID	GP* virus	2,0,0,0,0,0(0.16)	2,2,2,0,0,0(0.5)	4,4,4,4,2,2(1.66)
Gel-PPR+GP	$10^{3.5}$TCID	PPR virus	4,4,2,2,2,2(1.33)	4,4,4,4,4,4(2)	8,8,8,8,8,8(3)
	$10^{3.5}$ TCID	GP virus	2,2,2,2,2,2(1)	4,4,4,4,4,4(2)	8,8,8,8,8,8(3)
Gel-PPR+GP	$10^{4.5}$ TCID	PPR virus	4,4,2,2,2,2(1.33)	8,8,8,8,8,8(3)	16,16,16,16,16,16(4)
	$10^{4.5}$ TCID	GP virus	4,4,4,2,2,2(1.5)	8,8,8,8,8,8(3)	16,16,16,16,16,16(4)

CFT (Complement Fixation Test) serum antibody titers of goats vaccinated with three types of different vaccines. TCID= tissue culture infective dose, GP*= goat Pox, Gel= aluminum hydroxide gel. All gel based bivalent PPR+GPV vaccine contained different amount of immunogen that is $10^{2.5,}$ $10^{3.5\ and}$ $10^{4.5}$ TCID50 units/ml.

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