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*Full Length Research Paper*

# Study on immunogenicity of combined sheep and goat pox and peste des petitis ruminants vaccines in small ruminants in Ethiopia

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**The study was conducted at the National Veterinary Institute (NVI), Ethiopia, with the objective of evaluating the immunogenicity of combined peste des petits ruminants (PPR) and sheep and goat pox (SGP) vaccines under laboratory and field conditions for possible use of a cost efficient vaccine based disease control strategy. Vaccines of PPR and SGP were prepared using PPR75/1 (LK6 Vero74) and Kenyan sheep and goat pox (0180) strains separately as per NVI vaccine production protocol. Sera were collected from a total of 100 unvaccinated small ruminants (40 sheep and 60 goats) and screened for the presence of PPR and SGP virus specific antibodies. Then, animals that were found negative for the diseases were grouped, vaccinated, and clinically monitored by regular observations and by recording daily rectal temperature. Serum samples were collected at regular intervals for determination of sero-conversion. Challenge test was conducted separately for PPR and sheeppox vaccines and in combination of the two. Generally, vaccination against PPR and sheep pox using both combined and one vaccine alone conferred good protective efficacy. Both sheep and goats vaccinated against sheep pox using both combined vaccine and sheeppox vaccine alone were protected against challenge infection. The PPR trial showed that 50% of the control groups showed rise in body temperature and 37.5% of them died of PPR, while for sheep pox challenge group, 52.6% (n = 10/19) from the unvaccinated group showed typical pox lesion (fever, nodules) and 15.8% (n = 3/19) died of sheep pox. The serological test result show that vaccinated goats with PPR were sero-positive up to 75% and it reached 100% immediately post challenge, while sheep pox vaccinated groups showed up to 1/128 sero-positivity in serum neutralization test.**

**Key words**: Sheep and goatpox, PPR, vaccine, seroconversion, Ethiopia.

## INTRODUCTION

Ethiopia has a very huge livestock population of which 46 million of sheep and goats represent almost 60% (Mitiku, 2004). The sheep and goats supply more than 30% of the domestic meat consumption and generate cash and hard currency from export of meat, live animals and skin (Alemayehu and Fletcher, 1991). Although the contribution of sheep and goats to the Ethiopian agrarian economy is significant, small ruminant production in the country is not commensurate with the demand. This is because the sub-sector is constrained by various factors such as animal diseases, traditional system of management, inadequate feed supply and poor genetic potential. Infectious diseases of small ruminants also cause substantial loss through morbidity and mortality. Peste des petits ruminants (PPR) and sheep and goat pox (SGP) are among the commonest of the diseases that affect small ruminants entailing a huge economic loss. The two diseases are Office International des Epizooties (OIE) listed trans- boundary diseases

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**Table 1.** Results of challenge with sheep pox virus in the study animals.

| Group | Number vaccinated | | | Animal with SPX lesion | | Animals that died with SPX | |
|---|---|---|---|---|---|---|---|
| | Goat | Sheep | Total | Number | Percent (%) | Number of death | Percent death (%) |
| SPX | 4 | 9 | 13 | 0 | 0.0 | 0 | 0.0 |
| PPR +SPX | 4 | 8 | 12 | 1 | 8.3 | 1 | 8.3 |
| Control | 6 | 13 | 19 | 7 | 52.6 | 3 | 15.8 |
| Naturally sick | 0 | 2 | 2 | 2 | 100.0 | 2 | 100.0 |
| Total | 14 | 32 | 46 | 10 | 21.7 | 6 | 13.0 |

affecting the economy of the country through limiting international trade of animals and animal products (OIE, 2008a, 2008b).

Sheep and goats are reared by poor developing countries in Africa including Ethiopia. The diseases are priority animal diseases to be considered in poverty alleviation policy in areas where they are endemic. Peste des petites ruminant (PPR) is a contagious viral disease caused by a *Morbillivirus* in the family *Paramyxoviridae*. The natural disease affects mainly goats and sheep, but it is usually more severe in goats where it causes heavy losses, and is only occasionally severe in sheep. Sheep and goatpox (SGP) are viral diseases of sheep and goats characterized by fever, generalized papules or nodules, vesicles (rarely), internal lesions (particularly in the lungs) and death. Sheep and goatpox are caused by strains of *Capripoxvirus* in the family Poxviridae, which can infect both sheep and goats (OIE, 2008a and 2008b).

Both PPR and SGP are endemic in Ethiopia and cause huge economic losses through high mortality and morbidity rates. Currently, the National Veterinary Institute (NVI) produces live attenuated vaccine for both diseases using PPR75/1 (LK6 Vero74) and Kenyan sheep and goat pox (0180) strains separately. The test results of the combination of these two diseases vaccines were reported by Chaudhary et al. (2009) and indicated that the vaccines did not interfere with each other in safety and immunogenicity. It was also reported that the combination of the two vaccines would minimize the cost of vaccination and discomfort to the animals associated with multiple injections. The NVI was produced combined rinder pest and contagious bovine pleuropneumona (CBPP) vaccine using $T_1$SR strain during the rinder pest campaign conducted in Ethiopia to combat these two important cattle diseases until rinder pest was eradicated.

The present study was, therefore, designed to systematically investigate the immunogenicity of combined PPR and SGP vaccines under laboratory and field conditions for possible use of combined vaccines for cost efficient vaccine based disease control strategy.

## MATERIALS AND METHODS

### Description of the study area

The present study was undertaken at the NVI, Debrezeit, from September 2010 to June 2011. Debre ziet is located 47 km South-East of Addis Ababa with an altitude of about 1,900 me above sea level. The area receives an annual rainfall of 1,115.6mm with two rainy seasons: March to May (short rainy season) and June through September (main rainy season). The average maximum and minimum temperature are 30.5°C and 8.5°C, respectively (NMSA, 2003).

### Vaccine preparation

Vaccines of PPR and SGP were prepared using PPR75/1 (LK6 Vero74) and Kenyan sheep and goat pox (0180) strains separately as per NVI vaccine production protocol. The safety, potency and the sterility of each vaccine was checked separately before and after freeze- drying. The two vaccines were combined during reconstitution for combined vaccination and were administered subcutaneously with a minimum of $10^{2.5}$ $TCID_{50}$ viruses per dose for each vaccine.

### Laboratory trial

A total of 40 sheep and 60 goats (unvaccinated) were purchased and numbered with animal tags. Sera were collected from each animal and screened for the presence of PPR and SGP virus specific antibodies. Animals that were found negative for the diseases were grouped and vaccinated with single and combined vaccine, clinically monitored by regular observations and by recording daily rectal temperature. Serum samples were collected at regular intervals for determination of sero-conversion.

### Challenge test

Challenge test was conducted separately for PPR and sheeppox vaccines. For sheep pox challenge test, two naturally sheep pox sick animals were purchased from Debre zeit town and housed with 13 animals vaccinated with SPX, 12 vaccinated with SPX+PPR and 19 negative controls (Table 1). The animals were observed for the development of specific disease symptoms and rectal temperature was recorded daily. For PPR challenge test, three naturally sick goats purchased from Afar area were mixed with 16 PPR vaccinated, 16 PPR + SPX combined vaccine, and 16 control animals and they were kept in the same house. Body temperature and any clinical signs were observed and recorded daily.

### Serology

Competitive ELISA based on the use of MAb anti-nucleoprotein and a recombinant nucleoprotein produced in *Baculovirus* was used as described by supplier manual (CIRAD). Briefly, the plate was

coated with PPR antigen by adding 50 µl diluted in phosphate buffered saline (PBS) in 1/1300 dilution rate and incubated for 1 h at 37°C.Then, the plates were washed three times in washing buffer and blot dried. About 45 µl of blocking buffer was added to all wells, and then 5 µl of blocking buffer was further added to the monoclonal control wells, 55 µl of blocking buffer to conjugated control wells, 5 µl of test sera to test wells, 5 µl of strong positive, weak positive and negative sera to control wells, and 50 µl of monoclonal antibody diluted 1/100 in blocking buffer to all wells except the conjugate control wells and incubated 1 hat 37°C. Then, the plates were washed three times and blot dried. 50 µl of anti-mouse conjugate 1/1000 in blocking buffer was added and incubated at 37°C for 1 h. Then, the plate was washed three times with washing buffer. 50 µl substrate/chromogen solution was added and kept for 10 min in the dark place. Finally, stop solution was added and read with ELISA reader at 492 nm. The ODs of all samples including the controls were calculated and are expressed as the percent inhibition (PI) as follows: PI= 100-[(OD of the wells/OD of the monoclonal wells]. Those greater or equal to 50% were considered as positive.

#### Virus neutralisation test (VNT)

A test sera were titrated against a constant titre of capripoxvirus 100 $TCID_{50}$ [50% tissue culture infective dose] and the test was done using 96- flat-bottomed tissue culture grade microtitre plates based on the procedure described on OIE (2008a).

#### PCR tests

##### *PCR sheep pox*

Tissue samples, pox nodule, collected from sheep pox sick animals were tested with polymerase chain reaction PCR as described on OIE (2008b). DNA was extracted by using the DNeasy Tissue Kit (Qiagen, Germany according to the manufacturer's instructions. The PCR tests were performed in premix reaction tubes. The primers used were: #lsd43U-GTGGAAGCCAATTAAGTAGA, #lsd383U- CCCAATATTCTGCTGCTCTT the reaction conditions were: 95°C for 1 min, 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 70 s and a final extension step of 72°C for 5 min. When the #lsd383U and #lsd1263 pair was used, the reaction conditions were: 95°C for 1 min, 35 cycles of 94°C for 30 s, 49°C for 30 s, 72°C for 70 s and a final extension step of 72°C for 5 min.

##### *RT-PCR for PPR*

Tissue samples, and mouth lesion, collected from PPR sick animals were tested with reverse transcription-polymerase chain reaction (RT-PCR) as described on OIE (2008a). RNA easy kit (Qiagen Inc., Valencia, CA, and USA) was used for the extraction of the RNA of the virus from the tissue samples as per manufacturer's protocol. The purity and quantity of RNA was determined by UV-spectrophotometer. The cDNA synthesis was carried out using hexa-nucleotide random primer (MBI Fermentas, Glen Burnie, MD, USA) as follows: 10 µl of RNA along with 1 µl of random primer was heated at 70°C for 5 min and snap-cooled on ice and 1 µl of RNAsin (Promega, Madison, WI, USA) was added to the denatured RNA. To this, primer/template mixture, reverse transcription buffer (Promega) containing 25 mm Tris HCl pH-8.3, 50 mm KCl, 2.5 mm $MgCl_2$ and 100 ml each of dNTPs in a total volume of 20 µl was added along with 200 U of reverse transcriptase (RT) enzyme (MMuLV) (Promega) and incubated at 37°C for 90 min. The synthesized cDNA (5 µl) was used for the amplification of coding sequences of N, M, F and H genes, using virus-specific primers commercially synthesized from Metabion GmbH, Germany. The 50 µl reaction mix contained 10 pmole each of the primers in the presence of 25 mm Tris HCl (pH 8.3), 50 mm KCl, 2 mm $MgCl_2$, 100 µm each of four dNTPs and 2.5 units of high-fidelity Taq DNA polymerase (MBI Fermentas), and the following cycling conditions was used for amplification. The initial denaturation at 95°C for 2 min (1 cycle); 94°C for 1 min; 55°C for 1 min; 72°C for 2 min (35 cycles); and final extension at 72°C for 10 min (1 cycle) was followed to amplify the specific PCR products, which were later analyzed in 1% agarose gels stained with ethidium bromide along with standard MW DNA marker.

## RESULTS

### Post vaccinal reaction

The study animals were observed and their rectal temperatures were recorded daily. Reaction at the site of inoculation, clinical signs, or rise of body temperature was, however, not observed in all groups of animals.

### Challenge test

Challenge study for sheep pox was observed for 38 days post challenge. Clinical signs of sheep pox disease were not observed in goats and sheep vaccinated with sheep pox vaccine. Only one sheep showed sheep pox lesion and also finally died of sheep pox infection from the combined sheep pox and PPR vaccinated group while 52.6% (n = 10/19) from the unvaccinated group showed typical pox lesion (fever, nodules) and 15.8% (n = 3/19) died of sheep pox. Two of the naturally sick sheep purchased for contact challenge also died (Table 1, Figure 1).

### PPR vaccine trial

After three months of vaccination, vaccinated and unvaccinated control goats were challenged with contact challenge by mixing three PPR sick goats purchased from Afar. The goats were closely observed for the development of any clinical signs and daily body temperature was also measured. 50% of the control groups showed rise in body temperature, mouth lesion and diarrhoea and 37.5 % (n = 3) of them died of PPR (Figure 1). All of the naturally sick goats purchased for challenge also died. The vaccinated ones (PPR alone or combined PPR +SPX) did not die of PPR except but showed slight rise in body temperature for a few days (Table 2).

### Serological test results

#### *C-ELISA test result for PPR*

The serum samples collected were analyzed using c-ELISA

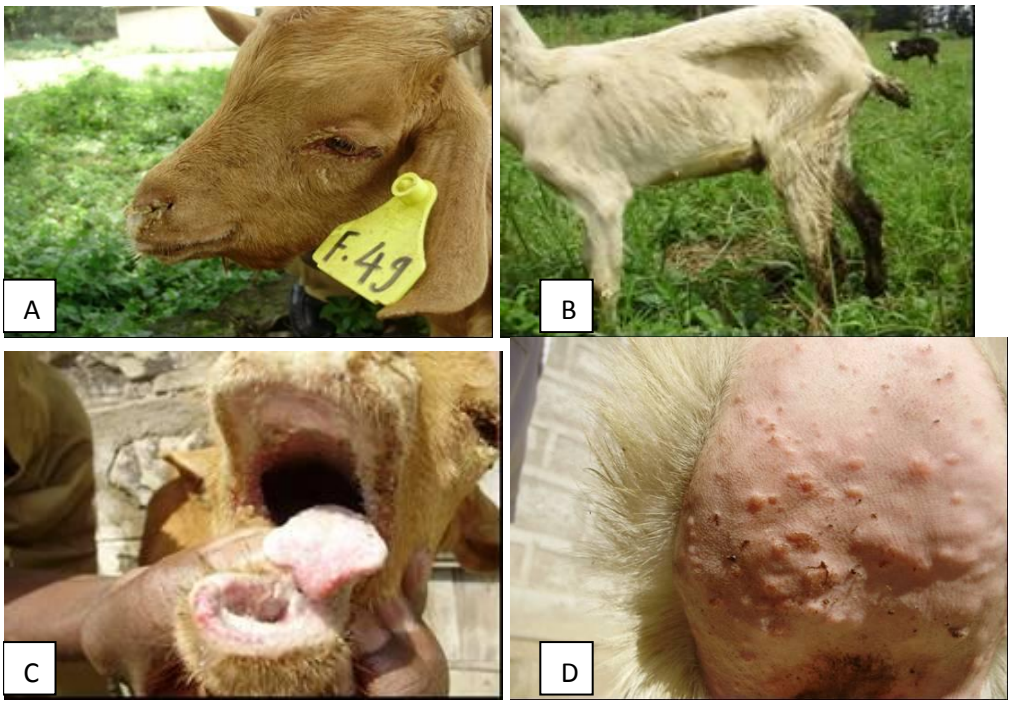


**Figure 1.** PPR and sheep pox lesions observed during challenge. Picture A, B, C (PPR lesion) and D (pox lesion).

**Table 2.** Results of challenge test in goats challenged with PPR virus.

| Group | Number vaccinated | Fever | Cough | Diarrhea | Mortality | Mortality (%) |
|---|---|---|---|---|---|---|
| PPR | 16 | 2 | 0 | 0 | 0 | 0 |
| PPR +SPX | 16 | 3 | 0 | 0 | 0 | 0 |
| Control | 16 | 8 | 4 | 4 | 3 | 37.5 |
| Natural sick | 3 | 3 | 3 | 3 | 3 | 100 |
| Total | 51 | 16 | 8 | 7 | 6 | 37.5 |

for the presence of PPR specific antibodies and the mean percent of inhibition (PI) were found to be 58.1, 57.6, and 44.8% for PPR+SPX, PPR and the controls, respectively, at day 14 post vaccination, while the PI significantly increased for all groups post challenge (Figure 2). For proportion of positivity; 75 and 66.7% of goats were positive post vaccination for PPR+SPX and PPR vaccinated groups, respectively, while the percent positivity reached 100% post challenge for both groups (Figure 3).

### *VNT test result for sheep pox*

The serum samples collected at day 14 post vaccination were tested with VNT for the development of specific SPX virus antibodies. Those sheep and goats vaccinated with both SPX vaccine and SPX combined with PPR vaccine developed high titre of antibodies up to 1:128 neutralizing antibodies, while the unvaccinated ones were negative.

### PCR test results

Cell culture and PCR test were conducted to confirm whether the outbreak samples (those used for PPR and Sheep pox challenge) were PPR and sheep pox or not. The PCR test result indicates that samples collected from naturally sick goats and sheep used for challenging were positive both for PPR and Sheep pox respectively.

## DISCUSSION

The objective of the present study was to develop such a tool by developing combined vaccine against sheep and goat pox and PPR which is able to protect small ruminants against both PPR and sheep and goat pox


**Figure 2.** Mean percent inhibition of serological test post PPR vaccination and challenge.

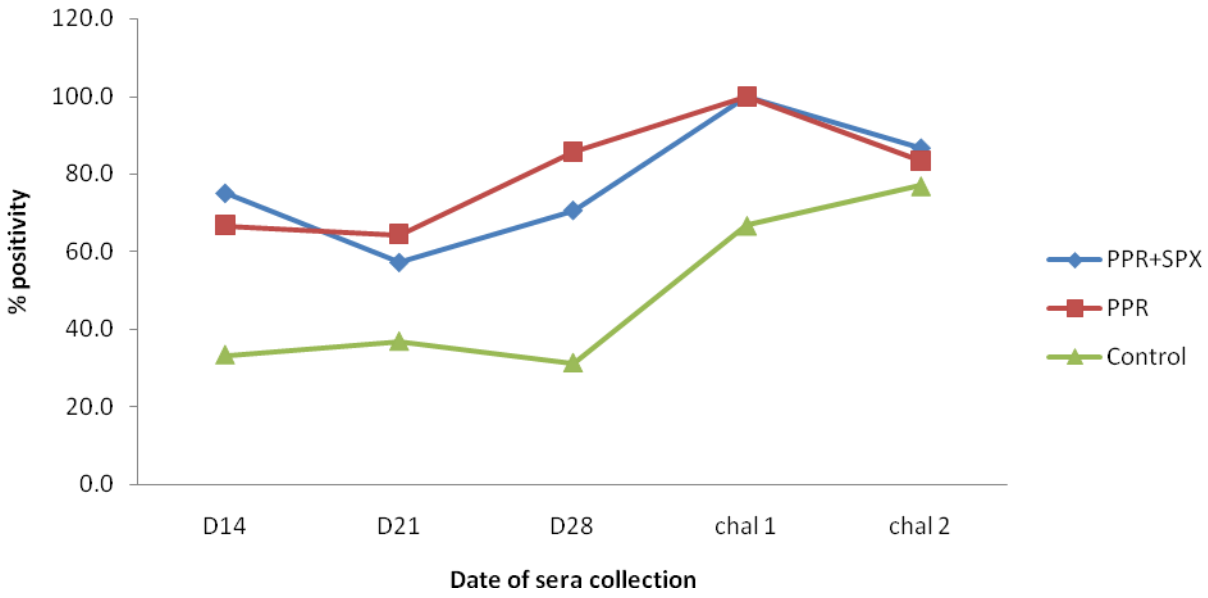


**Figure 3.** Percent of goat becoming sero-positive post vaccination and challenge.

simultaneously. Single vaccination covering both diseases can facilitate greater convenience, bring down the cost of vaccination significantly, and reduce stress to the animal and vaccination teams. This approach would also assist to a great extent in mass immunization programs as sometimes PPR can be a predisposing cause for sheep pox and vice-versa. In the present study, protective immune response to combined vaccine containing live attenuated strains of sheep and goat pox virus and PPRV was evaluated. Immunized animals remained apparently healthy without any signs of illness. These animals showed a transient marginal rise in temperature, an observation consistent with most of live attenuated viral vaccines.

The serological test result show that vaccinated goats were seropositive up to 75% and it reached 100% immediately post challenge, which indicated that even though the animals developed antibody below detectable levels, the animals remained protected against PPR infection. With combined PPR and sheep and goat pox vaccine using sheep and goats as experimental animals, PPRV antibody was detected on 14, 21, and 28 days

post immunization in sera of all vaccinated sheep and goats. Similar findings were reported by Hosamani et al. (2006) using bivalent PPR and goat pox vaccine and Singh et al. (2004) using PPR vaccine. Antibodies to sheep pox virus were not detectable in any of the group initially until day 7 post vaccination using serum neutralization test. A steady increase in antibody response was observed on day 14 post vaccination. These sheep and goats also showed a booster response when challenged on day 30 post vaccination using their respective contact challenge. A similar observation was reported by Bandyopadhyay et al. (1999).

There was no significant difference in humoral response between animals vaccinated using sheep pox or PPR vaccine separately or in combination. All control animals developed signs specific to disease after challenge. Sheep and goat pox vaccinated animals and those vaccinated with combined vaccines did not show any localized lesions at the site of inoculation. Similarly, PPR vaccinated and combined vaccine groups also resisted contact challenge infection. Thus, the vaccines when given alone or in combination at the recommended field doses conferred protection to homologous challenge. There was no untoward reaction when the combined vaccine was used at 100 field dose in safety test.

The efficacy of the combined vaccine in sheep has been evaluated; the findings indicate that combined vaccine induced protective immune response as evident from sero-conversion as well as protection on homologous challenge in sheep implying that both vaccine viruses did not interfere with the immunogenicity of each other. In support of the present observations, previous studies by Rajak et al. (2005) showed that PPR vaccine virus does not interfere with the immunogenicity to other unrelated antigens.

Thus, the present investigations shows that both sheep and goat pox and PPR vaccine viruses are compatible with each other making the live combined vaccination which provides a feasible approach for controlling these infections. It is presumed that the combined vaccine produced on a large scale and applied in the field will be a very cost effective alternative to individual vaccination strategies in developing countries.

In conclusion, both sheep pox and PPR vaccines when given alone or in combination at the recommended field doses conferred protection to homologous challenge. The finding suggests that combined vaccine is equally potent and safe to use. Thus, the current investigations show that sheep pox and PPR vaccine viruses are compatible with each other making the live combined vaccination a feasible approach for controlling these infections.

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