Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 23(3) September-December 2025]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology
Pantnagar, U.S. Nagar; Uttarakhand, Website : gbpuat.res.in/PJR

PANTNAGAR JOURNAL OF RESEARCH

Vol. 23(3) September-December, 2025

CONTENTS

Evaluation of neutralizing post-vaccination antibody response against Peste des petits ruminants virus in Pantja goat breed of Uttarakhand, India

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ABSTRACT: Pantja is a newly registered medium sized, dual purpose goat breed found in the Tarai region of Uttarakhand, India. As meat and milk of goat is highly preferred by Indians, its farming is vital for the agro economy in Indian subcontinent. Studies have been done on the growth performance of Pantja goat. However, studies on it's immunological parameters like response to vaccination and immunity against diseases is lacking. As it is a newly registered breed and considered as an important source of income for the poor people living in the Tarai region of Uttarakhand, it's characterization against robustness for various diseases is also important. This study was carried out to study the post vaccination antibody response in Pantja breed of goat against PPR. To evaluate the antibody response generated after vaccination by PPRV/Sungri/96 strain, five goats were vaccinated by one ml reconstituted PPRV/Sungri/96 vaccine sub-cutaneously and serum samples were tested at different time intervals for studying the antibody response. The development of PPRV neutralizing antibodies was measured using a virus neutralization test (VNT). All five animals exhibited neutralizing antibody response from seven to ten days post vaccination and maintained protective antibody titre upto 91 days post vaccination. The present study shows that Pantja goat exhibits strong protective titre as early as seven days post vaccination against PPR.

Keywords: Eradication, neutralising antibody, post-vaccination antibody response, Pantja goats, PPR-CP

Peste des petits ruminants (PPR) is a highly contagious viral disease affecting small ruminants and various wild Capra species. It is caused by the *Peste des petits ruminants virus* (PPRV), a member of the *Paramyxoviridae* family. First identified in the early 1940s in Côte d'Ivoire, West Africa, PPR has progressively spread, and is now endemic across large parts of sub-Saharan Africa, North Africa, the Middle East, and much of Asia, excluding South-East Asia (Banyard *et al.*, 2010; Parida *et al.*, 2016, 2015).

In India, the economic impact of PPR is significant. Estimated losses in sheep and goats due to the disease amount to INR 5041.5 million and INR 11074.6 million, respectively—mortality accounting for 77% and 73% of the losses, and morbidity contributing 23% and 27% (Govindaraj *et al.*, 2016). The burden of PPR can be substantially reduced through successful eradication. In pursuit of this goal, the World Organization for Animal Health (OIE) and the Food and Agriculture Organization (FAO) have set a target to eradicate PPR globally by 2030. A key component of this effort is the deployment of effective vaccines supported by reliable diagnostic tools. In endemic regions, PPR vaccines have been widely used via subcutaneous administration in sheep and goats, demonstrating considerable success (Parida *et al.*, 2015; Sen *et al.*, 2010). Recent studies have confirmed that these vaccines provide protection against field strains from all four genetic lineages (Hodgson *et al.*, 2018).

In Uttarakhand, the PPR-Control Programme (PPR-CP) was initiated during the second phase in 2014–15 (Balamurugan *et al.*, 2016a). Pantja is a newly registered, medium-sized, dual-purpose goat breed indigenous to the Tarai region of Uttarakhand, India. While goat meat and milk are highly valued in Indian cuisine, goat farming plays a critical role in the agrarian economy of India and the broader Indian subcontinent. Pantja goats are especially noted for

their deer-like morphological features and thrive in the hot and humid conditions of the Tarai region in Uttarakhand and neighboring districts of Uttar Pradesh.

Studies have been made on the growth performance of Pantja goat (Khadda *et al.*, 2017). However, studies on it's immunological parameters like response to vaccination and immunity against diseases is lacking. As it is a newly registered breed and forms an important source of income for the poor people living in the Tarai region of Uttarakhand, it's characterization against robustness for various diseases is also important.

This study was carried on to study the post vaccination antibody response in Pantja breed of goat against PPR. The neutralizing antibody titre was determined against PPR in vitro using Virus Neutralisation Test.

MATERIALS AND METHODS

Animal experiment

To study the post vaccinal antibody response against PPR, five kids of Pantja goat breed (Animal number G665, G666, G668, G669, & G672) each 4 months old were vaccinated sub-cutaneously with one ml PPR vaccine and 3 ml blood was withdrawn from the jugular vein of each kid at various time points. Rectal temperature was measured each time before withdrawal of blood.

The blood was collected at 0 dpv (day post vaccination), 5 dpv, 7 dpv, 10 dpv, 12 dpv, 15 dpv, 18 dpv, 21 dpv, 28 dpv, 35 dpv, 42 dpv, 49 dpv, 56 dpv, 63 dpv, 70 dpv, 77 dpv, 84 dpv& 91 dpv. The blood samples were processed to collect the serum. These serum samples were tested by competitive ELISA to measure the kinetics of antibody generated after vaccination. 0 dpv denotes blood collected before vaccination.

Ethics Statement

The above animal experiment has been approved by the Institutional Animal Ethics Committee (IAEC).

Competitive ELISA for PPRV Anti-N Antibodies

Serum samples were tested by competitive ELISA (c-ELISA) kit (ID Screen® PPR Competition, France) for detection of anti-PPRV antibodies in terms of competition percentage (S/N %). The test was performed as per the manufacturer's guidelines. First, all the reagents used in the test were equilibrated at room temperature prior to performing the assay. The microplates were already precoated with purified recombinant PPR nucleoprotein (NP) by the manufacturer. To the precoated plates, 25 µl samples (to be tested) and the 25 µl controls (positive and negative control) diluted in dilution buffer were added to each well (25 µl each well). The plate was gently tapped to ensure that all the fluid in the well was mixed properly. The controls were tested in duplicate wells and test samples in single well. The plate was covered with aluminium foil and incubated for 45 min ± 4 min at 37^0 C (± 3^0 C). Each well was washed 3 times with wash buffer (provided by manufacturer) by flooding each well upto the top. The wash buffer was discarded by inverting the plate and tapping it over the towel. After washing of wells, diluted (1/10) anti-NP-HRP conjugate was added 100 µl to each well. Contents of the wells were mixed by gentle tapping the sides of the plate. The plate was covered with aluminium foil and incubated for 30 min ± 3 min at 21^0C (± 5^0C). Plates were washed, dried and 100 µl of substrate was added to each well. Plate was incubated for 15 min ±2 min at 21^0C (± 5^0C) in the dark. The reaction was stopped by stop solution by adding 100 µl to each well. The plate was read at 450 nm wave length in the ELISA reader (Epoch, BioTek, USA) using Gen5, BioTek version 3.04 software.

The results were expressed as competition percentage (S/N %) which was calculated as follows:

$$S/N\% = \frac{\text{OD sample}}{\text{OD negative control}} \times 100$$

The results were interpreted as follows-
Positive : S/N % $\leq$ 50 %
Doubtful: S/N % >50 % or $\leq$ 60%
Negative: S/N % > 60 %
Samples categorized as doubtful were retested by IDvet ELISA.

Cells and Viruses

The Vero cell line (ATCC-CCL 81) was used for performing the virus neutralization test (VNT) and was procured from Indian Veterinary Research Institute (IVRI), Mukteshwar, Uttarakhand. Vero cells were cultured in Eagle's minimum essential medium (EMEM) HIMEDIA, India with 10% fetal bovine serum (HIMEDIA, India) and antibiotics (Streptomycin and Penicillin, HIMEDIA, India).

Virus Neutralizing Antibody Titer

The development of PPRV-neutralizing antibodies was assessed using a virus neutralization test (VNT), following previously established protocols (OIE, 2019). The 50% neutralization endpoints were calculated using the Reed and Muench method (Reed and Muench, 1938). A neutralizing titer greater than 10 was considered positive. Each test was conducted at least twice, and the average of the two results was used for further analysis. Neutralising antibody titre was estimated at 0dpv, 7dpv, 10dpv, 14dpv, 21dpv, 28dpv, 56dpv, and 84 dpv.

Statistical Analysis

Post-vaccinal antibody response was analyzed using mixed models in which the vaccine used and time were fixed factors, and individual animals were random factors. One-way ANOVA followed by Sidak multiple comparison test was performed using GraphPad Prism version 9 for Windows (GraphPad Software, La Jolla California, CA, USA, www.graphpad.com). Graph was plotted using MS excel 2007.

RESULTS AND DISCUSSION

Clinical Observations

During the acclimatization period (the first week after arrival), the animals' rectal temperatures remained within the normal range of 38.5 to 39.5°C (data not shown). After vaccination, none of the animals exhibited any adverse reactions, and there were no PPRV-specific clinical signs observed in the vaccinated animals.

Antibody Response against the Viral Nucleocapsid Protein generated in Pantja goats vaccinated against PPRV/Sungri/96

In our study, to study the kinetics of anti-PPRV antibody development, serum samples were collected at 2-3 days interval for upto three weeks (21 dpv) and thereafter at weekly time point. All the animals were seronegative at 0dpv and 5 dpv. At 7 dpv the range of the antibody response lies both above and below 50 cut-off value (Fig 1-5). Two goats (Animal number G-666 and G-668) at 7 dpv started exhibiting detection of anti-PPRV antibodies (Fig 2 & 3) and three goats (Animal number G-665, G-669, & G-672) at 10 dpv (Fig 1, 4 & 5). This study corroborates with the study of Mahaptra and colleagues (Mahapatra *et al.*, 2020) in which the antibody response against PPRV nucleoprotein after vaccination with Sungri/96 in British goats has been found to develop at 7 dpv in all animals. However, intriguingly in our study it was found that the commencement of antibody response post PPRV vaccination by Sungri/96, may vary from seven to ten days. In a study by Hodgson *et al.* (2018) (Hodgson *et al.*, 2018) after vaccination by Sungri/96, the antibody response against PPRV N protein has been found to develop from 14 dpv. The overall antibody response in all five animals was not significantly different (p=0.5352). In other studies (Begum *et al.*, 2016; Gowane *et al.*, 2016; Hodgson *et al.*, 2018; Mahapatra *et al.*, 2020) post vaccination antibody response have been studied at a weekly interval.

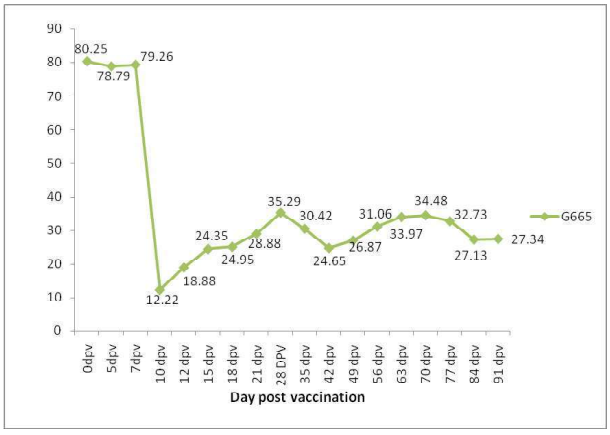

Fig. 1: Competitive-ELISA result at different time periods (Mentioned in graph). Antibody response in goat (G 665) vaccinated against PPRV. Result is given in terms of competition percentage (S/N%)

The one animal (Animal number G-668) was weak positive on 7dpv (S/N 46.84). However, other animal (Animal number G-666) was strong positive (S/N 12.65) in antibody response. The overall antibody response in all five animals was not significantly different (p=0.5352). The mean antibody response of all five animals is depicted in Fig. 6. All the animals were strongly seropositive upto 91 days post vaccination.

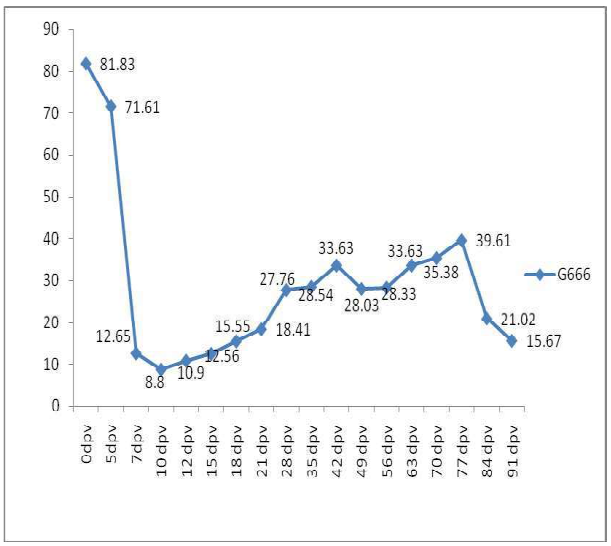

Fig. 2: Competitive-ELISA result at different time periods (Mentioned in graph). Antibody response in goat (G 666) vaccinated against PPRV. Result is given in terms of competition percentage (S/N%)

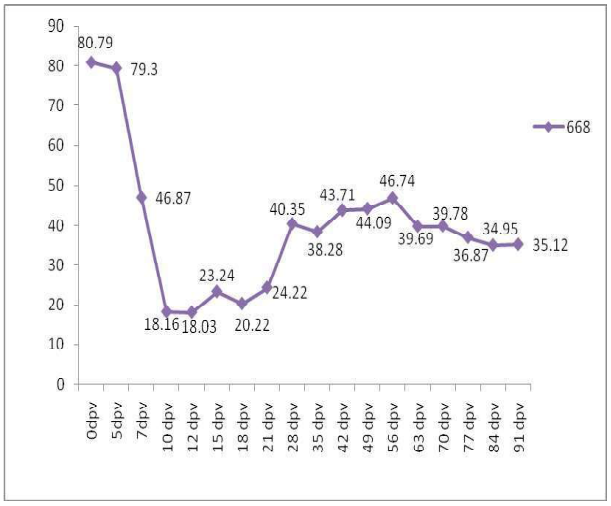

Fig. 3: Competitive-ELISA result at different time periods (Mentioned in graph). Antibody response in goat (G 668) vaccinated against PPRV. Result is given in terms of competition percentage (S/N%)

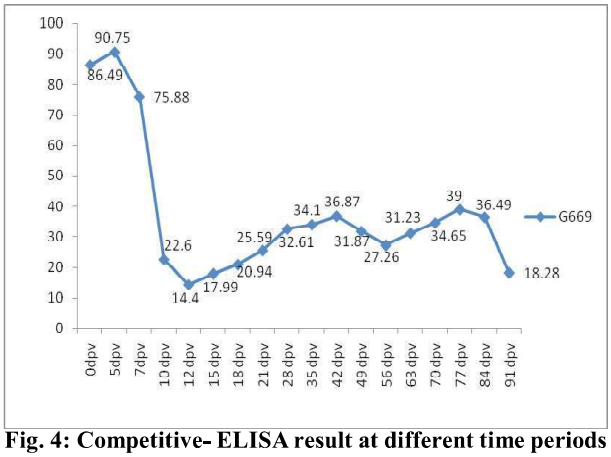

Fig. 4: Competitive- ELISA result at different time periods (Mentioned in graph). Antibody response in goat (G 669) vaccinated against PPRV. Result is given in terms of competition percentage (S/N%)

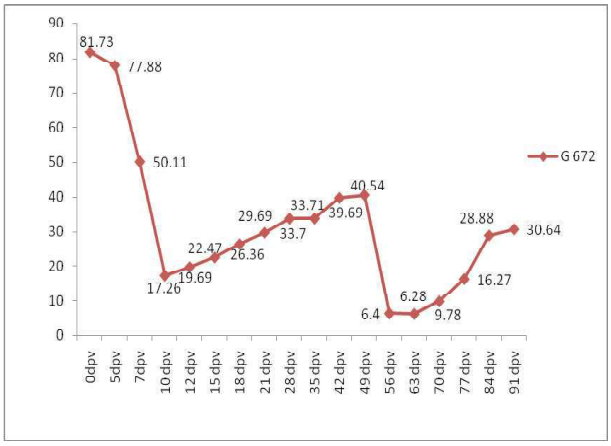

Fig. 5: Competitive-ELISA result at different time periods (Mentioned in graph). Antibody response in goat (G 672) vaccinated against PPRV. Result is given in terms of competition percentage (S/N%)

Table 1: Post-vaccination neutralizing antibody titre of goats vaccinated with PPRV Sungri-96. dpv=Days post-vaccination after the animals were vaccinated with PPRV Sungri-96. Numbers 665,666, 668, 669 & 672 denote the animal number vaccinated with PPRVSungri-96 Neutralising antibody titre is denoted in the columns against days post-vaccination and animal number

dpv	665	666	668	669	672
0 dpv	0	0	0	0	0
7 dpv	0	15	15	0	0
10 dpv	15	15	25	15	20
15 dpv	30	120	50	15	40
28 dpv	240	240	200	100	200
56 dpv	240	240	200	120	200
84 dpv	240	240	200	120	240

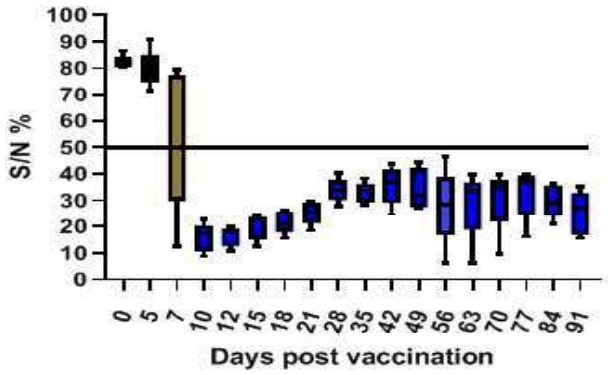

Fig. 6: Box plot for Competitive- ELISA result at different time periods for 5 Pantja Goats. Result was interpretated as in terms of competition percentage (S/N%). **The data are presented as box-and-whisker plots, in which the bars span the minimum and maximum values of five animals. The central horizontal line in each box shows the median.**

Neutralizing Antibody Titres

After confirming seropositivity by c-ELISA kit the serum samples were tested for development of protective neutralizing antibody response. The neutralizing antibody response was detected by VNT. All animals were negative in protective antibody titre on 0 dpv. Two animals 666 and 668 exhibited protective neutralizing antibody titre from 7 dpv onwards whereas other three animals 665, 669 and 672 exhibited neutralizing antibody titre from 10 dpv onwards (Table 1, Fig 7). At 7 dpv both animal number 666 and 668 exhibited neutralizing antibody titre of 15. Remaining animals didn't exhibit neutralizing antibody response at 7 dpv. From 10 dpv animal number 665, 669 and 672 started exhibiting neutralizing antibody response, with titre ranging from 15 to 20. From 10 dpv the neutralizing antibody response started increasing steadily for all animals and attained peak at 28 dpv, with the titre ranging from 100 to 240. (Fig 7, Table 1). Thereafter, strong protective antibody titre ranging from 120 to 240 was maintained in all animals upto 91 dpv.

The study was in corroboration with earlier studies (Satav *et al.*, 2020) in which the antibody titre of 1:128 post 21 dpv was detected after immunizing with PPRV Sungri 96 vaccine. Singh *et al.*, 2004 study reported an antibody titre of 1:256 at 21 dpv after vaccinating with Sungri-96. In a study by Holzer and colleagues (Holzer *et al.*, 2016) the post vaccination antibody response by Sungri-96 was elicited at 14dpv and peaked at 28dpv.

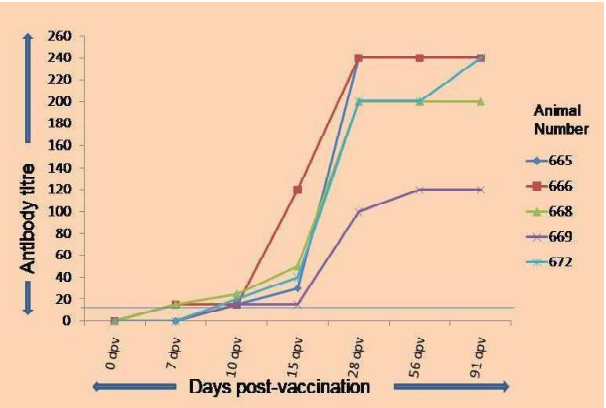

Fig. 7: Post-vaccination neutralising antibody titre in Pantja goats elicited after vaccination against PPR. The horizontal line indicates the cut-off antibody titre (10) above which the antibody titre is considered as protective

CONCLUSION

The first nine days after vaccination are crucial as during this time the antibody response against PPRV may not develop in some animals. Therefore, introduction of new animal into the flock from outside and movement of goats for grazing with new animals must be restricted at least for first ten days after vaccination. The current study depicts that the Pantja goat evokes a strong protective immunization response post vaccination as elicited by a strong protective antibody titre. However, as the first antibody appears at 7-10 days, the goats shall remain for PPRV infection upto seven days.

ACKNOWLEDGEMENTS

The funding for this work was acquired from Directorate of Research, GB Pant University of Agriculture & Technology, Pantnagar, Uttarakhand.

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Received: October 22, 2025
Accepted: November 13, 2025