J. Zoo Biol. 07 (01) 2024. 29-34 DOI: 10.33687/zoobiol.007.01.5083

Available Online at EScience Press
Journal of Zoo Biology
ISSN: 2706-9761 (Online), 2706-9753 (Print)
https://esciencepress.net/journals/JZB

# CHARACTERIZATION OF MURINE MONOCLONAL ANTIBODIES BY HYBRIDOMATECHNOLOGY FOR THE PESTE DES PETITS RUMINANTS (PPRV)VACCINE

**[a,b]Tahira Kamal*, [b]Saeed-ul-Hassan, [b]Fariha Hasan, [a]Ghulam Muhammad Ali**

*[a] National Institute of Genomics and Advanced Biotechnology (NIGAB), National Agriculture Research Centre (NARC), Islamabad 44000, Pakistan.*

*[b] Quaid-i-Azam University, Islamabad, Pakistan.*

**ARTICLE INFO**

**Article History**
Received: February 16, 2024
Revised: May 29, 2024
Accepted: August 12, 2024

**Keywords**
Monoclonal antibodies
Isotyping
Peste des petits ruminants
Indirect ELISA
SDS-PAGE, Pakistan

**ABSTRACT**

Peste des petites ruminants (PPRV) is an acute and highly contagious viral disease of sheep and goats. It continues to be a major cause of economic losses worldwide. The causative agent is from the genus *Morbillivirus*. It is very much antigenically similar to the rinderpest virus. In this study, we developed specific monoclonal antibodies (mAbs) against PPRV. These monoclonal antibodies will be used for the future development of diagnostic assays against PPRV. Monoclonal antibodies are widely used in molecular biology and biomedical research. However, the generation of these molecules is complicated and time-consuming. The production of PPRV-specific mAbs involved immunizing BALB/c mice with a commercially available PPRV vaccine. Spleenocytes were then fused with myeloma cells (SP2/0) using polyethylene glycol fusion (PEG fusion). A total of five monoclonal antibodies (mAbs) out of seven were found positive in IELISA These are 1A-1, 2A-5, 3B-5, 4C-2, and 5C-5 raised against the PPRV vaccine. These were developed by the CBIS method (cell-based immunization and screening), that is fusion of myeloma cell line SP2/0 with mice spleenocytes, collected from purified PPRV vaccine immunized mice. These mAbs were then characterized by Indirect ELISA and western blot analysis(SDS-PAGE). One of the positive hybridoma clones (3B-5) secreting mAbs belonged to the IgG class, which was purified using a Protein A- Plus spin kit (Thermo Scientific) and showed a single band in SDS-PAGE. The mAbs named, (3B-5) represented a positive, 70-kDa protein band in SDS-PAGE analysis. The mAbs established in this study are useful for studying the interaction between PPRV and its target cell. The current study effectively characterized the mAbs and found one positive 3B-5 was proved to be efficient for detecting antibodies against PPRV in sheep and goat sera and would use to develop diagnostic assays for PPRV in Pakistan. This is the first ever report from Pakistan, that evaluate the practical approach for the characterization of monoclonal antibodies against the PPRV virus.

**Corresponding Author: Tahira Kamal*
*Email: tahirakhurram3@gmail.com*


## INTRODUCTION

The Peste des petits ruminant virus (PPRV) is a highly contagious and economically significant viral disease affecting small ruminants (Dong *et al*., 2020; Singh, *et al*., 2014). The PPR virus (PPRV) is a member of the *Morbillivirus* genus in the Paramyxoviridae family (Vinayagamurthy *et al*., 2020; Balamurgun *et al*., 2022). All members of Paramyxoviridae are closely related and have similar antigenic characteristics. PPRV virus has a mortality ratio of 25.76% in adults (over one year) and 27.39% in goats, under one year (Abubakar *et al*., 2008). It was believed that PPRV primarily affected small

ruminants, including sheep and goats (Banyard *et al*., 2010). PPRV has been reported in Pakistan since 1991 with initial epidemics in the Punjab region being characterized by using PCR in 1994 (Amjad *et al*., 1996; Rind *et al*., 2023).

Even though our raised mAbs were exceptionally specific when analyzed by indirect ELISA. However, commercially available cELISA kits (Libeau *et al*., 1995, Rojas *et al*., 2019) do not cross-react significantly with other Morbilliviruses. Specific mAbs against the PPRV N-protein are important. These are needed to develop reliable diagnostic techniques, as shown by Libeau.,*et al*. (2014), Kinimi *et al*. (2021), and Balamurgun *et al*. (2020). This research was conducted to characterize the N-protein of developed mAbs, against the PPRV vaccine virus from Pakistan.

Hybridoma technology was invented by Köhler and Milstein in 1975, and it has remained unaltered for 40 years. Monoclonal antibodies are essential for flow cytometry, cell sorting, and immunofluorescence (Van Regenmortel *et al*., 2000; Harms., *et al*., 2023). They also provide diagnostic and therapeutic options for disease surveillance and treatment. This study aimed to characterize the developed monoclonal antibodies. They were made to detect antibodies against the PPRV virus. These mAbs were the first ever raised in Pakistan against the Peste des petits ruminant virus (PPRV). It also monitored the antibody response level *in vitro*, cell culture supernatants from developed hybridomas. Two fusions were performed using a commercially available vaccine antigen for immunizing mice. The production of seven mAbs is described in this report. The virus-neutralizing ability was evaluated for the selected mAbs. Subsequent evaluation of mAbs with indirect ELISA and isotyping of developed positive mAbs were performed.

## ETHICAL CONSIDERATIONS

An Ethical Certificate was approved for this study by the Institutional Biosafety Committee: (IBC Reference No.: NIGAB/NARC/02/05-01-2020) National Agriculture Research Center (NARC)Islamabad.

## MATERIALS AND METHODS

In this study, we characterized seven mAbs, developed by the CBIS method. A PPRV vaccine antigen was used for immunization of mice. The developed monoclones were evaluated and characterized using the following methods: Indirect enzyme-linked immunosorbent assay (IELISA), Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Positive mAbs were also isotyped for further evaluation (Kinimi *et al*., 2021).

Myeloma cells, Sp2/0, were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Healthy Balb/c mice were purchased from the animal facility NUST, Islamabad. Production of monoclonal antibodies (mAbs) was performed as per the protocol (Singh *et al*., 2004) which included the use of Balb/c mice (4- 6 weeks old), 20-25g weight at the time of immunization. These were screened by Indirect ELISA for antibodies in sera. A commercially available vaccine with 100 TCID50 was used for the immunization of the two Balb/c mice. Myeloma (SP2/0) cells were cultured for the fusion procedure. The PEG-induced fusion of Myeloma (SP2/0) and spleen cells was conducted (Singh *et al*., 2004). Repeatedly, indirect enzyme linked immuno-sorbent assay (IELISA) was used to screen growing hybridoma clones (Singh *et al*., 2004; Fayaz *et al*., 2022; Mallick *et al*., 2023). The wells of 96 wells cell culture plates, showed positive clones and were re-cultured using a limited dilution method for single-cell cloning and sub-cloning of selected cells. (Le *et al*., 2020).

The obtained positive monoclonal antibodies (mAbs) were assessed for their antigenic structure and purity using SDS-PAGE. Evaluating their ability to detect the N-protein. Western blotting was used. Lysates prepared in 1% Triton X-100 buffer was centrifuged to remove cell debris. We separated 10 µg of proteins using a 20% polyacrylamide gel for 3 hours at 120V. Then, we stained them with a colloidal Coomassie G-250 solution for 60 minutes at 25-28°C. Subsequently, we transferred them to 0.45 µm nitrocellulose membranes for 2 hours at 100 V. Finally, we developed the blots with a 0.05% diaminobenzidine (DAB) solution (Sigma, St. Louis, MO, USA) in PBS containing 1% hydrogen peroxide. After three washes with PBS-T, membranes were washed with deionized water. Images were then captured using ImmunoStar LD (FUJIFILM Wako Pure Chemical Corporation).

Finally, we determined the isotype of the generated mAbs. To do this, we used the Pierce TM Rapid ELISA Mouse mAbs Isotyping Kit, as directed by the manufacturer.

## RESULTS

### Development of Hybridomas

Following the HAT selection protocol, hybridoma

development was observed within 10- 15 days, marked by the emergence of cell clones. This critical phase was monitored using a microscope. We examined each well in a 96-well plate. We used a 10X objective lens and a 10X eyepiece. This allowed us to identify monoclones precisely. The successful clones were then annotated directly on other plates for subsequent analyses.

**Identification of PPRV-Specific Monoclonal Antibodies via IELISA**

The harvested culture supernatant from individual wells was analyzed. We used an Indirect Enzyme-Linked Immunosorbent Assay (IELISA), which targeted antigen-specific signals for Peste des Petits Ruminants Virus (PPRV). This method facilitated differentiating positive and negative clones based on their antigenic specificity. Notably, five out of seven, monoclonal antibodies (mAbs) 1A-1, 2A-5, 3B-5, 4C-2, and 5C-5 showed significant reactivity in IELISA. This indicates their potential for application in PPRV diagnostics (Figure 1).

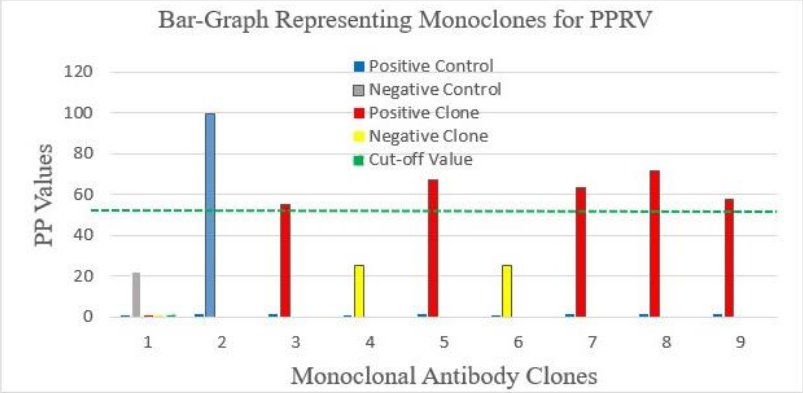


Figure 1. Bar Graph of Monoclones representing Seven monoclones; Five positive monoclones(red), negative clones (yellow), analyzed by IELISA.

**Virus Neutralization Testing (VNT) Performance**

Virus Neutralization Testing (VNT) against a known PPRV antigen was used. The aforementioned mAbs showed different levels of neutralization efficacy. Specifically, mAbs 3B-5 and 5C-5 achieved complete neutralization of the PPRV antigen, with endpoint dilutions recorded at 1:16 and 1:32, respectively. This finding underscores the potential of these mAbs as therapeutic candidates

**Characterization through SDS-PAGE**

Subsequent characterization of all seven specific hybridoma clones developed for PPRV was conducted by SDS-PAGE analysis, with particular attention to mAbs 3B-5 and 5C-5 Figure 2. The 3B-5 mAbs exhibit a strong affinity for the N-protein of PPRV. This was evidenced by its specific band patterns at 70KD in the gel. This finding indicates its potential utility in specific diagnostic assays aimed at N-protein detection.

**Isotyping of Monoclonal Antibodies**

Further analysis included the isotyping of the developed monoclonal antibodies named mAbs 3B-5 and 5C-5, which were determined to be of the IgG subclass. The presence of a positive band for the IgG heavy chain was demonstrated by isotyping assays. This provides valuable information for the future application of these mAbs in diagnostic and therapeutic settings (Figure 3).

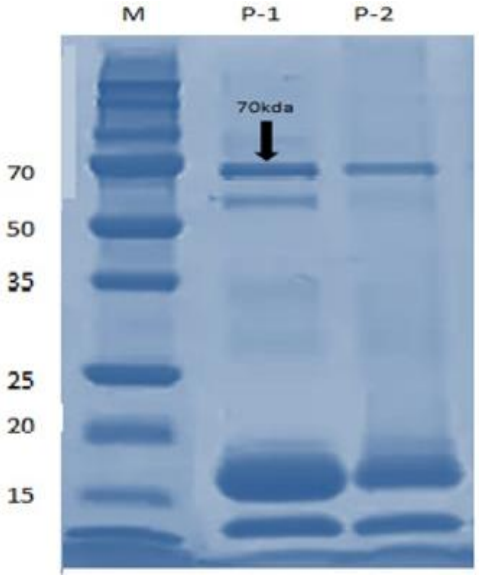


Figure 2. SDS-PAGE results depicting the specific reactivity of mAbs 5C3 and 5D4 towards the N-protein of PPRV.

These results highlight the successful development and characterization of monoclonal antibodies against PPRV. They showed that the antibodies could be used in diagnostic assays. They also support further research into the immune response to PPRV infection.


Figure 3. Results from the isotyping assays, illustrating the IgG1 heavy chain specificity of mAbs 3B-5 (5C3).

**DISCUSSION**

The development of monoclonal antibodies (mAbs) against the Peste des Petits Ruminants Virus (PPRV) represents a significant advancement in the control strategies for PPRV diseases. This study successfully produced a set of positive mAbs: 1A-1, 2A-5, 3B-5, 4C-2, and 5C-5. These mAbs showed promising results in IELISA, virus neutralization tests (VNT), and isotyping. The results highlight their potential utility in diagnosing and studying PPRV.

The ability of mAbs 3B-5 and 5C-5 to achieve complete neutralization of the PPRV antigen, in VNT is particularly noteworthy. This suggests that these antibodies could play a crucial role in the development of therapeutic strategies against PPRV. They could also be important in the development of diagnostic assays. The specificity of mAbs 3B-5 for the N-protein of PPRV is confirmed by SDS-PAGE. This further supports their potential use in diagnostic assays that required high specificity.

The positive monoclonal antibodies (mAbs) produced by Hosny, exhibited complete or incomplete neutralization in VNT against PPRV isolate. (Hosny *et al*., 2021). Two of his selective monoclones completely neutralized the PPRV antigen in VNT. It is known that, that all mAbs developed in a single study may not be characterized for any specific viral protein (Fayaz *et al*., 2022).

As for our developed mAbs, 3B-5 and 5C-5 have complete virus-neutralizing ability. Others have only a partial ability to neutralize 100 TCID50 of PPRV when analyzed by a virus neutralization test(VNT) supported by (Tully *et al*., 2023). Those mAbs being applied in a cELISA kit are used to detect PPRV disease (Tully *et al*., 2023; Liu *et al*., 2023; Singh *et al*., 2004). Other developed mAbs are not successful in establishing their protein specificity. However, the mAbs 3B-5 represented higher titers, more specific to anti-N-protein, when detected by SDS-PAGE in (Figure: 3). The mAbs 3B-5 uniquely showed a strong affinity at 70 kDa for the N-protein of PPRV in SDS-PAGE. These results revealed that mAbs 3B-5 belonged to the N-protein (nucleoprotein), presenting a protein band of approximately 70 kDa. In SDS-PAGE, the other four positive mAbs did not exhibit any band, confirming the specificity of mAbs 3B-5 for N- protein of PPRV. Previously, (Balamurgun *et al*., 2021) utilized these types of mAbs for PPRV in sero-surveillance and sero-monitoring of PPRV, all around India.

The isotyping of mAb 3B-5 as IgG offers more insight into the immune response against PPRV. It provides a basis for further research into how antibodies neutralize the virus. Understanding the isotypes of antibodies involved in the neutralization of PPRV can aid in the design of vaccines. It can also help in creating therapeutic antibodies tailored to elicit a specific immune response. The monoclonal antibody (mAb) 3B-5, developed in this study, was isotyped as I Gg. It showed a positive band for IgG in (Figure 3) heavy chain isotypes and other bands were negative for other subclasses like kappa and lambda proteins.

**CONCLUSION**

Monoclonal antibodies (mAbs) 3B-5 and 5C-5 completely neutralized the Peste des Petits Ruminants Virus (PPRV) strain in Virus Neutralization Testing (VNT). Indirect ELISA revealed significantly higher reactivity of all five MAbs compared with the antibody and antigen controls combined. mAbs 3B-5(IgG), showed a strong affinity for the 70-KD N-protein of PPRV in SDS-PAGE analysis. The mAbs analyzed in this study could be used for rapid and effective PPRV diagnostic assays in the future. These mAbs can be used to study immunological studies for PPRV and differentiate from other *Morbilliviruses*. However, in Pakistan, further investigations are required.

These investigations will be used to produce effective mAbs against PPRV, which further assist to develop specific and sensitive diagnostic assays for PPR disease, in Pakistan. They will develop specific and sensitive diagnostic assays for PPRV diagnosis.

**ACKNOWLEDGEMENT**

This study was funded by Grant No: NIGAB/AS-029/2019/PARC, received from the Agri Linkage Programme of Pakistan Agriculture Research Council (ALP-PARC).

**AUTHORS CONTRIBUTION**

Tahira Rana**;** Conceptualization, Methodology, writing – original draft, Writing – review & editing, Project administration, Data curation, Formal analysis, Ghulam Ali; Funding acquisition, Resources, Saeed-Ul-Hassan Khan; Supervision, Fariha Hasan; Visualization, Review & editing.

**CONFLICT OF INTEREST**

The author(s) declare(s) that there is no conflicts of interest regarding the publication of this article.

**REFERENCES**

Van Regenmortel, M.H., 2000. The recognition of proteins and peptides by antibodies. Journal of immunoassay 21, 85-108.

Rojas, J.M., Rodríguez-Martín, D., Avia, M., Martín, V., Sevilla, N., 2019. Peste des petits ruminants virus fusion and hemagglutinin proteins trigger antibody-dependent cell-mediated cytotoxicity in infected cells. Frontiers in Immunology 9, 3172.

Kinimi, E., Muyldermans, S., Vincke, C., Odongo, S., Kock, R., Parida, S., Mahapatra, M., Misinzo, G., 2021. Development of nanobodies targeting peste des petits ruminants virus: the prospect in disease diagnosis and therapy. Animals 11, 2206.

Dong, D., Zhu, S., Miao, Q., Zhu, J., Tang, A., Qi, R., Liu, T., Yin, D., Liu, G., 2020. Nucleolin (NCL) inhibits the growth of peste des petits ruminants virus. The Journal of General Virology 101, 33.

Libeau, G., Prehaud, C., Lancelot, R., Colas, F., Guerre, L., Bishop, D., Diallo, A., 1995. Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleobrotein. Research in veterinary science 58, 50-55.

Balamurugan, V., Varghese, B., SowjanyaKumari, S., Kumar, K.V., Muthuchelvan, D., Govindaraj, G., Suresh, K., Hemadri, D., Roy, P., Shome, B., 2022. Assessment of post-vaccination immune response to peste des petits ruminants virus in small ruminants in the central and western regions of India. VirusDisease 33, 413-421.

Hosny, W., Baheeg, E., Hassanein, S., Mohamed, S., 2021. Preparation of a house ELISA kit for detecting Peste des petits ruminants Virus (PPRV) antibodies. Benha Veterinary Medical Journal 41, 24-28.

Tully, M., Batten, C., Ashby, M., Mahapatra, M., Parekh, K., Parida, S., Njeumi, F., Willett, B., Bataille, A., Libeau, G., 2023. The evaluation of five serological assays in determining seroconversion to peste des petits ruminants virus in typical and atypical hosts. Scientific Reports 13, 14787.

Fayaz, A., Rajak, K.K., Kumar, A., Karki, M., Rai, V., Bhatt, M., Singh, R.P., 2022. Development and characterization of mouse monoclonal antibodies to canine morbillivirus. Biologicals 79, 19-26.

Balamurugan, V., Varghese, B., Kumar, K.V., Muthuchelvan, D., Dheeraj, R., Govindaraj, G., Suresh, K.P., Hemadri, D., Roy, P., 2020. Seroprevalence study of peste des petits ruminants in sheep and goats in the northern region of India. Veterinary World 13, 1573.

Liu, D., Li, L., Cao, X., Wu, J., Du, G., Shang, Y., 2021. Selection and identification of single-domain antibody against Peste des Petits Ruminants virus. Journal of Veterinary Science 22.

Singh, R., Saravanan, P., Sreenivasa, B., Singh, R., Bandyopadhyay, S., 2004. Prevalence and distribution of peste des petits ruminants virus infection in small ruminants in India. Rev Sci Tech 23, 807-819.

Balamurugan, V., Vinod Kumar, K., Dheeraj, R., Kurli, R., Suresh, K.P., Govindaraj, G., Shome, B.R., Roy, P., 2021. Temporal and spatial epidemiological analysis of Peste des petits ruminants outbreaks from the past 25 years in sheep and goats and its control in India. Viruses 13, 480.

Vinayagamurthy, B., Gurrappa Naidu, G., Roy, P., 2020. Peste des petits ruminant virus. Emerging and Transboundary Animal Viruses, 315-343.

Chen, S., Yang, F., Cao, W., Zhang, X., Liu, H., Zhang, K., Liu, X., Wang, J., Zheng, H., Zhu, Z., 2023. Peste Des Petits Ruminants Virus Nucleocapsid Protein Interacts

with Protein Kinase R-Activating Protein and Induces Stress Granules To Promote Viral Replication. Journal of Virology 97, e01712-01722.

Le, K., Tan, C., Le, H., Tat, J., Zasadzinska, E., Diep, J., Zastrow, R., Chen, C., Stevens, J., 2020. Assuring clonality on the beacon digital cell line development platform. Biotechnology Journal 15, 1900247.

Mallick, S., Singh, R.P., Biswal, J.K., Mohapatra, J.K., Rout, M., Samanta, R., Khulape, S.A., Ranjan, R., 2023. Production and characterization of monoclonal antibodies against foot-and-mouth disease virus serotype O and development of a sandwich ELISA for virus antigen detection. Veterinary Research Communications, 1-10.

Singh, B., Bardhan, D., Verma, M. R., Prasad, S., & Sinha, D. K., 2014. Estimation of economic losses due to Peste de Petits Ruminants in small ruminants in India. Veterinary World, 7-4.

Libeau, G.,2014. Current advances in serological diagnosis of peste des petits ruminants virus. Peste des petits ruminants virus, 133-154.

Harms, P. W., Frankel, T. L., Moutafi, M., Rao, A., Rimm, D. L., Taube, J. M., ... & Pantanowitz, L., 2023. Multiplex immunohistochemistry and immunofluorescence: a practical update for pathologists. *Modern Pathology*, *36*-7, 100197.

Abubakar, M., Ali, Q., Khan, H.A., 2008. Prevalence and mortality rate of peste des petitis ruminant (PPR): possible association with abortion in goat. Tropical animal health and production 40, 317-321.

Amjad, H., Forsyth, M., Barrett, T., Rossiter, P., 1996. Peste des petits ruminants in goats in Pakistan. The Veterinary Record 139, 118-119.

Banyard, A.C., Parida, S., Batten, C., Oura, C., Kwiatek, O., Libeau, G., 2010. Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. Journal of general virology 91, 2885-2897.

Rind, A., Abro, S., Kaleri, R., Solangi, G., Mangi, R., Memon, M., Bhuptani, D., Noor, S., Lanjar, Z., Mangrio, Z., 2023. Prevalence of the peste des pettitis ruminants in goat and sheep in District, Sanghar, Sindh. Pakistan. Journal of Innovative Sciences 9, 192-197.