Explor Anim Med Res,
Vol.11, Issue - 1, 2021, p. 24-29
DOI : 10.52635/EAMR/11.1.24-29

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

CHARACTERIZATION OF IVRI-CSF-BS CELL CULTURE ADAPTED VACCINE STRAIN FOR NEWCASTLE DISEASE VIRUS EXALTATION (END) AND CORRELATION OF VIRUS TITER BY END METHOD WITH FAT

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Received 27 May 2021, revised 04 June 2021

ABSTRACT: The classical swine fever (CSF) viruses are generally non-cytopathic in nature and hence titrations of these viruses are mostly done by immunological methods such as fluorescent antibody technique (FAT). Although FAT is a gold standard and virus titration can be done very accurately, it involves tedious steps and demand skilful technological interventions. Exaltation of NDV (END method) has been used in the past to detect some of the CSF viruses in cell culture wherein Newcastle Disease Virus (NDV) readily proliferates in presence of CSFV and produces cytopathic changes. In the present study, a newly developed CSF vaccine virus strain (IVRI-CSF-BS) has been characterized for NDV exaltation and the virus has been found to be END+. This feature has been exploited for titration of the vaccine virus in PK-15 cells. The virus titre obtained by the END method has been correlated with the titres obtained by FAT and a linear correlation has been established as the titre obtained by END method was always 2 $\log_{10}$ lesser than the FAT titre.

Key words: Classical swine fever, Newcastle disease virus, FAT, Exaltation.

INTRODUCTION

Classical swine fever (CSF) is a highly contagious transboundary disease (Blome *et al*. 2017) causing high morbidity and mortality in both feral and domestic pigs (Ganges *et al*. 2020). Although the disease is known since long and much effort has been put towards its control, it is still regarded as a major problem in pig industry. The virus is endemic in many countries and has the potential to cause re-emergence in disease-free areas.

The etiological agent of this disease belongs to genus Pestivirus under family Flaviviridae together with Bovine viral diarrhoea virus (BVDV) and Border disease virus (BDV) and all these viruses have common feature of persistence (Smith *et al*. 2017). A high standard laboratory diagnosis is a pre-requisite for rapid detection and efficient control of the disease. The well-established diagnostic methods of CSF such as virus isolation, FAT, Ag ELISA, RT-PCR, VNT, indirect and cELISA have been widely used as per recommendation of OIE terrestrial manual (OIE Manual 2019). Observing cytopathic changes in cell culture also provides researchers a rapid clue of the many human and animal viruses. However, with a very few exceptions, the vast majority of CSFV isolates are non-cytopathogenic and do not produce visible cytopathic changes (CPE) in cell culture (Gallei *et al.* 2008). Most of these non-CPE CSFV field isolates show enhancement of cellular changes in presence of NDV (exaltation) and this is exhibited as CPE (Aoki *et al*. 2004). It has been reported that some strains of CSFV, which do not show Exaltation by ND virus (END phenomenon), show intrinsic interference (Fukusho *et al*. 1976). The mechanism of END phenomenon is a result of suppression of IFN production (Toba and Matumoto 1969). The END method was originally developed by Kumagai *et al*. (1961) (for estimation of the virus) as test tube method using primary culture of swine testicular (ST) cells. Later on, Lai *et al*. (1978) established estimation by micro-method using ST cells which was further replaced with CPK porcine kidney cell strain by Komaniwa *et al*. (1981).

In the present study, a vaccine virus strain developed in the laboratory was END characterized in PK-15 cells.

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Subsequently, the CSF vaccine virus was titrated by the END method and the titres were correlated with that obtained by FAT method. A significant correlation of the two methods would replace the tedious application of FAT with the END method in the laboratory.

MATERIALS AND METHODS

Cell

The stable porcine kidney cell line PK-15 (ATCC, USA), free of mycoplasma and ruminant pestivirus, was used in this study. The cells were propagated in Eagle's Minimum Essential Medium (HiMedia, India) supplemented with 10% foetal bovine serum (Invitrogen, USA) under 5% CO2 tension and 100 % relative humidity.

Viruses

A recently developed classical swine fever cell culture vaccine virus (IVRI-CSF-BS) was used in the study. It is an Indian strain of CSFV which is cell culture adapted by serial passaging in porcine cells (Manu 2018) and is reported to be a high yielding virus (Pachauri *et al*. 2020).

The Newcastle disease virus (NDV) used in this study was kindly provided by M/s. Hester Biosciences, Ahmadabad on request. The virus is a live vaccine virus containing 1000 doses of LaSota strain where each dose contains NLT 10^6 EID_{50} ND virus.

Characterization of CSF virus for exaltation of NDV:

Fresh monolayer of PK-15 cells was sub-cultured, and the cell concentration was adjusted to 5 x10^5 cells ml^{-1}. The cells were seeded @ 400 ml in each well of a 24 well microtiter TC plate and simultaneously infected with 100 ml of CSF freeze dried vaccine virus reconstituted in 1ml EMEM-SFM keeping few uninfected cell control wells. The plate was incubated at 37°C for 66 hours and the CSFV infected cells were re-infected with 1ml of serially diluted ND virus (10^{-1} dilution = 10^5 EID_{50} and 10^{-2} dilution = 10^4 EID_{50}) after removing the old medium from each well. Some CSFV infected wells were not infected with NDV as CFV controls, and some cell controls were infected only with NDV as NDV controls. The cells were again incubated and observed till 8th day under light microscope. The culture medium was then discarded, and cells were stained with crystal violet for 30 minutes after fixation with 3% paraformaldehyde for 20 minutes. Cell culture well plate was then dried and observed under microscope.

Same experiment was conducted by infecting NDV to PK-15 cells on the same day of CSFV infection.

Virus titration by FAT

Titre of the CSFV used in END assay was determined using FAT protocol followed in the laboratory (Dhar *et al*. 2008) with minor modifications and viral infective titre was determined.

Virus titration by END method

The END assay, which was used for END+ virus titration, was performed as micro-titre method in a 24 well tissue culture plate. Briefly, 400 ml cells (from 5 x 10^5 cells ml^{-1} suspension) were seeded into each well. Tenfold serial dilution of CSFV were made and 100 ml of each dilution was added to each well barring the NDV and cell control wells. The plate was then incubated for 2 days at 37 ^{0}C, 5% CO2 and culture fluid were aspirated. One ml of 10^{-2} diluted NDV was added and incubated up to eight days. The cells were then examined for appearance and enhancement of cytopathic effect, if any, under light microscope. The viral titre was estimated based on the highest dilution of END+ IVRI-CSF-BS strain yielding CPE upon NDV super-infection.

RESULTS AND DISCUSSION

END characterization of CSF virus

Clear cut cytopathic changes were observed following addition of NDV into CSFV infected PK-15 cells. Infected cells showed rounding, ballooning, polykaryocyte formation, vacuolation and even cell detachment (Fig. 1). All the control wells [cell control Fig. 1 (a), CSFV control Fig. 1 (b), NDV control Fig. 1 (c)] were negative for presence of any such changes. NDV infection which was given on the same day of CSFV infection did not produce any relevant result. So, NDV infection given on 2 days post infection (d.p.i) of CSFV infection was followed for rest of the experiments. The virus infective titre, observed from cellular changes, was very low in the initial days; however, changes became prominent from sixth day onwards. So, the vaccine virus IVRI-CSF-BS used in the study was designated as END Positive (END+).

Infective titer correlation

Virus infective titre was determined by the morphological changes in the cells. All wells inoculated with 10^{-1} dilution of NDV including 10^{-1} NDV control showed cell mortality and turbidity due to toxic content of the egg based NDV vaccine and hence, this initial dilution was excluded, and other higher dilutions were considered.

In virus titration by FAT, detection of green fluorescence was observed up to 10^{-7} dilution, which is

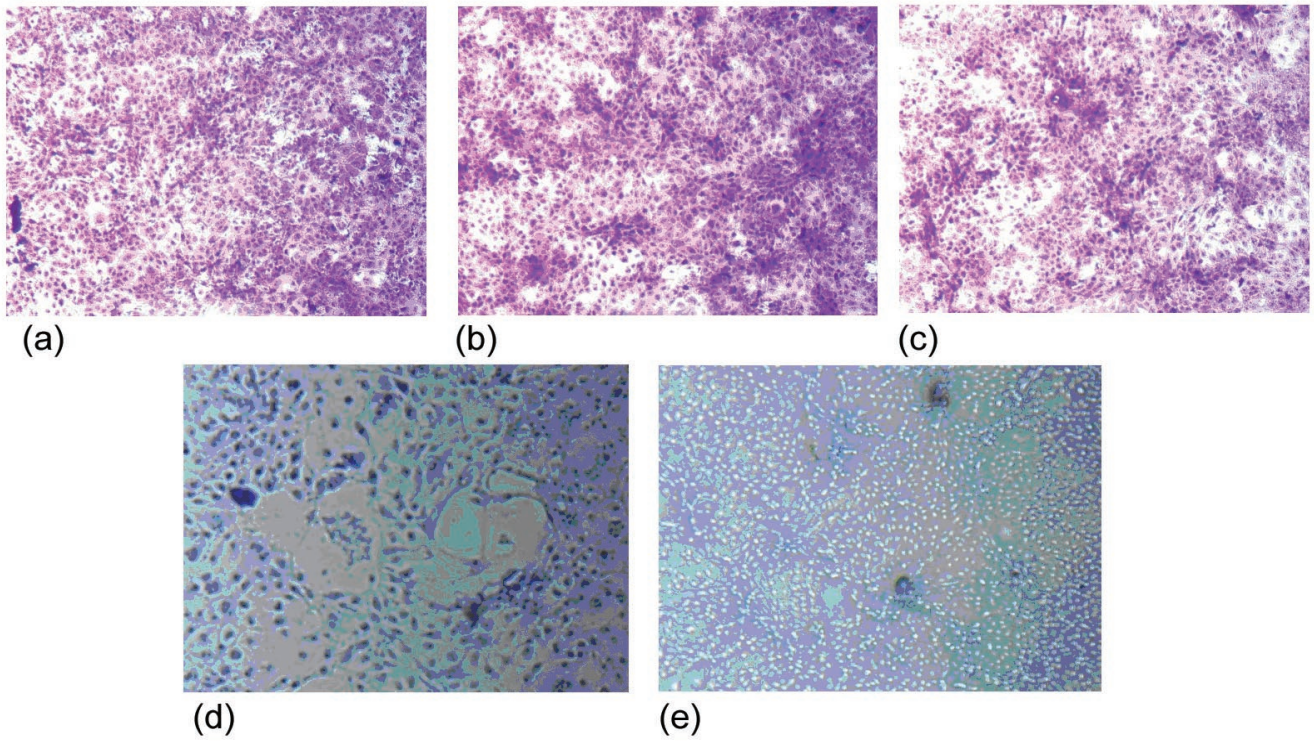

Fig. 1. END characterization of CSFV and cell staining by crystal violet. No changes were seen in cell control (a), CSFV control (b) and NDV control (c), whereas CSF infected and NDV super-infected cells showing ballooning and polykaryocyte formation (d) and vacuolation (e).

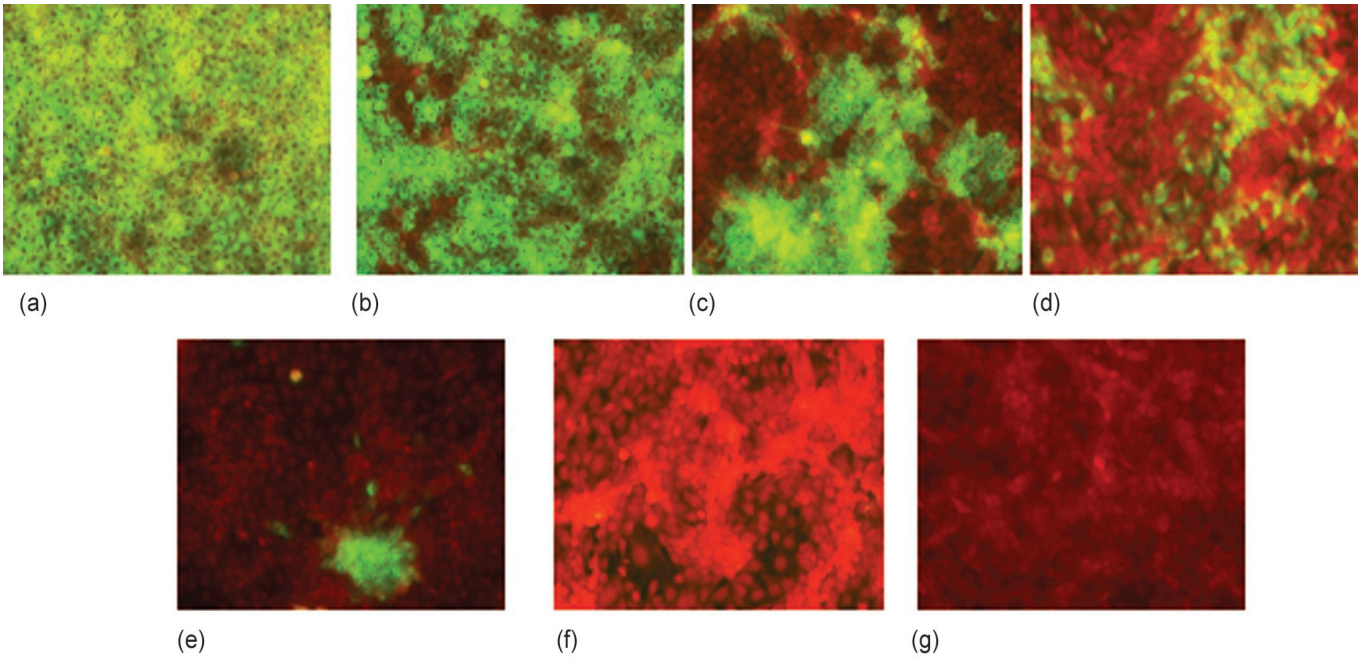

Fig. 2. CSFV titration by FAT. (a) to (e) are cells infected with virus dilutions from cells from 10^{-3} to 10^{-8} respectively. Green fluorescence was observed up to the dilution 10^{-7}. No fluorescence in cells infected with 10^{-8} dilution (f) as well as in the uninfected cell control (g).

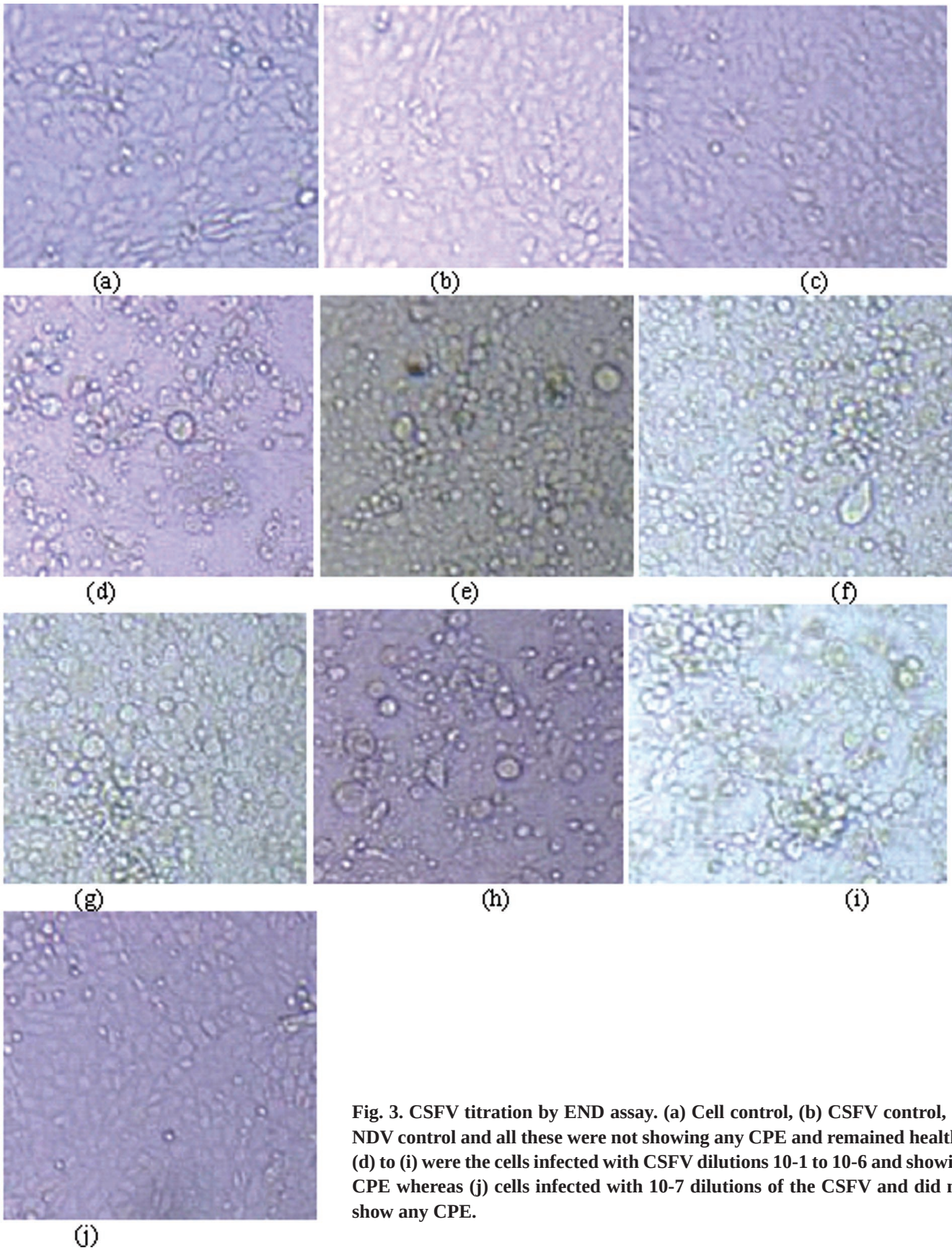

Fig. 3. CSFV titration by END assay. (a) Cell control, (b) CSFV control, (c) NDV control and all these were not showing any CPE and remained healthy. (d) to (i) were the cells infected with CSFV dilutions 10-1 to 10-6 and showing CPE whereas (j) cells infected with 10-7 dilutions of the CSFV and did not show any CPE.

indicative of presence of virus. Hence, FAT titre of the virus was calculated at $10^{8.5}$ $TCID_{50}$/ml (Fig. 2).

In END assay, clear and distinguishable morphological changes were observed in the cells infected with CSFV up to 10^{-6} dilution whereas the cells infected with 10^{-7} dilution and the controls (cell control, CSFV control and NDV control) did not show any such changes (Fig. 3). Hence, the infective titre determined by this assay was $10^{6.5}$ $TCID_{50}$/ml.

The infective titre determined by END method was $10^{6.5}TCID_{50}$/ml whereas the titre of the same virus in FAT was $10^{8.5}$ $TCID_{50}$/ml. So, the infective titre determined by END titration protocol was $2\log_{10}$ titre less than the actual FAT titre. Repeated experiments showed the similar results. Thus, although the END method was little less sensitive than FAT, it can be used to determine the infective titre of CSFV in PK-15 cells.

In the present communication, we report that the IVRI-CSF-BS vaccine virus is END positive and produces CPE by exaltation with ND virus which is of similar findings with the previous studies where some non-CPE CSF viruses were reported to be END+ (Kumagai *et al*. 1961). As already reported by previous researchers, accumulation of NS3 protein could induce cell apoptosis, which may be the underlying reason of exaltation (Xu *et al*. 2007). Aoki *et al*. (2004) had reported that the NS3 protein was strongly detected at 48 hrs after NDV inoculation, but the NS3 signal strength was increased in cells at 72 hrs after inoculation when CPE became evident. So, the absence of CPE, in the initial days of super infection in our experiment may be due to low level of NS3 in PK-15 cells. We also state an interesting finding that the NDV exaltation occurred when cells were infected with NDV after two days of CSFV infection whereas such exaltation didn't occur when PK-15 cells were infected simultaneously with CSFV and NDV. In case of same day infection with NDV and CSFV, enough amount of IFN might not have been suppressed, driving the cellular defences to remain active and not allowing NDV to produce CPE in CSFV infected cells (Toba and Matumoto 1971).

In our study, the END assay has been used for titration of CSF virus and compared with the gold standard, *i.e*., FAT. Though sensitivity of the END method is little lesser than the FAT method, its linear correlation with FAT titre can guide us to determine the actual virus titre. It is also evidenced in the work of Kumagai *et al.* (1961) that the infective titre determined by their method was less than swine inoculation method. This finding is in complete agreement with our experiment.

CONCLUSION

Any non-CPE viruses are difficult to work with and CSFV is not an exception. Although FAT is gold standard for its detection and titration, the test is not without many disadvantages such as labour intensive, requiring serious cell culture interventions etc. Our finding is that, NDV exaltation can be very well used for CSF virus titration, although the sensitivity is less.

ACKNOWLEDGEMENT

We appreciate the kind co-operation of Indian Veterinary Research Institute in providing funding and laboratory facilities. We would like to thank Dr. J. K. Pal for providing ND virus and Dr. Srikant Ghosh, Head, Division of Parasitology, IVRI, Izatnagar for providing fluorescent microscope facility.

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Cite this article as: Mahapatra CS, Dhar P (2021) Characterization of IVRI-CSF-BS cell culture adapted vaccine strain for Newcastle Disease Virus Exaltation (END) and correlation of virus titre by END method with FAT. Explor Anim Med Res 11(1): 24-29. DOI : 10.52635/EAMR/11.1.24-29