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Evaluation of Reactivity Profile of Non-Structural Protein C of Peste-Des-Petits-Ruminnats Virus

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Peste-des-petits ruminants (PPR) is an economically devastating viral disease of sheep and goats. In the present study, we have evaluated the reactivity profile of the recombinant non-structural protein C of PPR virus with vaccinated and convalescent goat sera in an indirect ELISA. The antibody against this protein is getting produced in both vaccinated as well as in convalescent animals. Further, we evaluated the antibody production kinetics in vaccinated goats and found that antibodies against this protein is getting produced from the 14th day of post-vaccination and persists till the 40th day of the study period. The data generated in this study will be useful for the development of DIVA vaccine and diagnostics against PPR.

Introduction

Peste-des-petits ruminants (PPR) is a viral disease of small ruminants caused by PPR virus. The international agencies FAO and OIE target the progressive control and eradication of this disease by 2030 (Parida *et al.,* 2015). The disease can occur in many forms like peracute, acute, and in-apparent forms and the morbidity and mortality and case fatality rates vary accordingly. Generally, the clinical symptoms are more severe in goats than in sheep (Sen *et al.,* 2010). The etiological agent, PPR virus belongs to the genus *Morbillivirus* of the family *Paramyxoviridae.* The genus *Morbillivirus* harbors many important pathogens like rinderpest in cattle, measles in humans, Canine distemper in Canines and morbilliviruses of marine animals. In the similar line of global rinderpest eradication, now FAO and OIE target the global eradication of PPR by 2030. Similar to rinderpest, this virus also occurs as single

serotype and genetically it can be classified into four distinct lineages (I, II, III, and IV) based on F gene (Shaila *et al.,* 1996) and N gene (Kwiatek *et al.,* 2007). Occurrences of all four lineages have been reported in many countries of Africa whereas only lineage IV has been reported across Asia (Banyard *et al.,* 2010; Parida *et al.,* 2015) with an exception of lineage II vaccine virus involvement in a outbreak in wild ruminants in China (Zhou *et al.,* 2017). The virus genome encodes for six structural (N, P, M, F, H & L) and two non-structural proteins (C & V) (Bailey *et al.,* 2005).

PPR situation in India is recently reviewed by our group and the outbreaks occur in all seasons (Muthuchelvan *et al.,* 2015). With the availability of live attenuated PPR vaccine and MAb based competitive and sandwich ELISA, the Govt. of India has launched a national control program on PPR in 2010 which resulted in significant reduction of PPR outbreaks in some states. However, the protective herd immunity has yet to be established for the whole country (Muthuchelvan *et al.,* 2014). The present vaccine being a live attenuated vaccine is expected to induce antibodies against all the viral proteins. Therefore, differentiating the infected from the vaccinated animals (DIVA) is difficult. Developing DIVA enabled vaccine and diagnostic assay will save money and labor during the eradication efforts.

The non-structural proteins – C&V are encoded in the P ORF. The C protein is generated from an alternate start codon (Bailey *et al.,* 2005; Muthuchelvan *et al.,* 2006). It is a small basic nonphosphorylated protein with a molecular weight of 19-21 kDa. Two primary functions of C protein have been reported namely, prevention of host innate immunity and control of viral RNA synthesis (Garcin *et al.,* 1999). It augments the maturation of infectious particle, has an antagonistic action against IFN and modulates RNA polymerase activity (Horvath, 2004). This protein can occur in both nucleus and cytoplasm of infected cells in Measles Virus and only in the cytoplasm of rinderpest virus (Patterson *et al.,* 2000) and blocks the type I interferon in rinderpest virus (Boxer *et al.,* 2009). Earlier we have reported the prokaryotic expression and confirmation of this protein (Bhadouriya *et al.,* in press). In the present study, we have evaluated the reactivity of this protein in an indirect ELISA. The data generated in this study will be useful at the time of formulation of DIVA vaccine and diagnostics.

Serum samples having the serum neutralization (SNT) titer ranged from 1:8 to 1:256 from the vaccinated animals (n=20) or convalescent sera (n=20) available in the laboratory were used for checking the reactivity. The sera with SNT titre <1:2 were used as negative control. The serum samples (*n* = 300) collected randomly from sheep/goats from different places of the country and were also included. To determine the time course of antibody production against this protein, we have vaccinated six animals with PPRV (Sungri/96) and collected the sera on 0, 7th, 14th 28th and 40th-day post vaccination.

A chequer-board titration was performed for optimization of antigen concentrations and antibodies dilutions (Bhadouriya *et al.,* in press). The indirect ELISA was optimized in-house, in brief; all the steps were carried out in 50 µL reaction volumes and incubated at 37 °C. After each incubation step, plates were washed three times with100 µL /well PBS containing 0.05% Tween-20 (PBST). Recombinant C protein @ 100 ng/well, was suspended in carbonate-bicarbonate buffer (pH 9.6). Wells were blocked with PBS containing 5% skimmed milk powder and 2% chicken serum. The goat sera samples were diluted @ 1:10 in blocking buffer.

Fig.1 Reactive pattern of recombinant PPRV C protein with vaccinated (n=20), convalescent (n=20) and negative sera (n=20) in an indirect ELISA

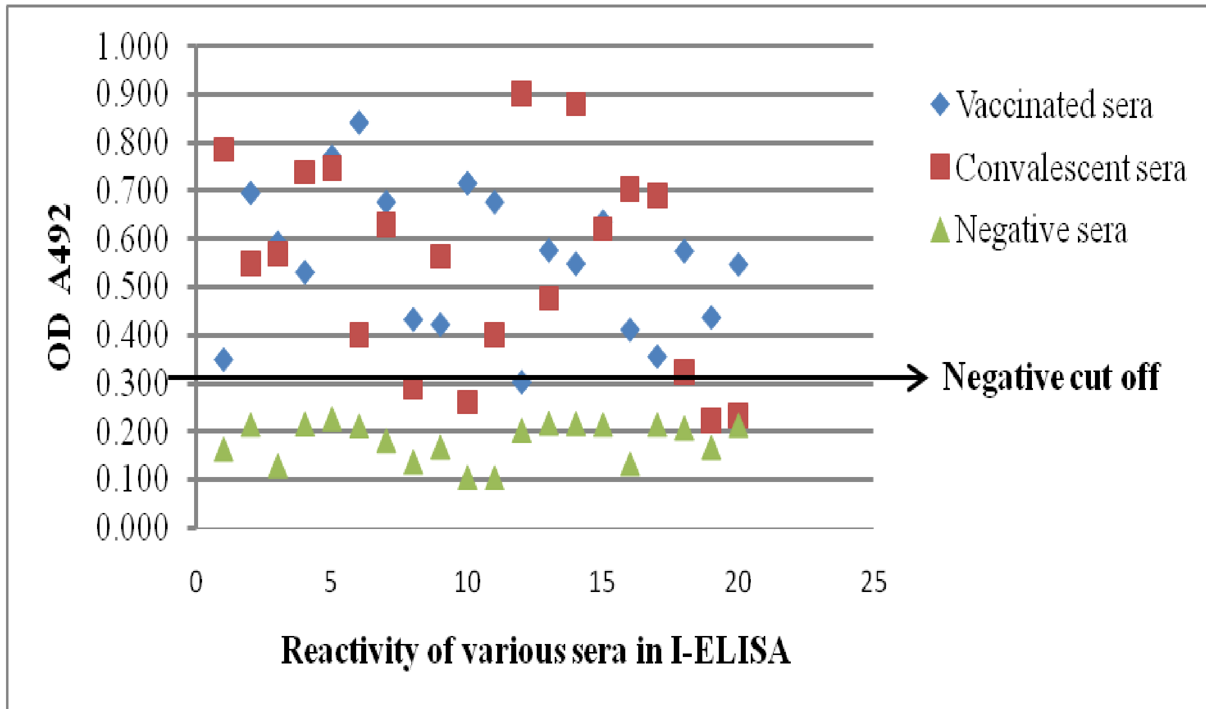
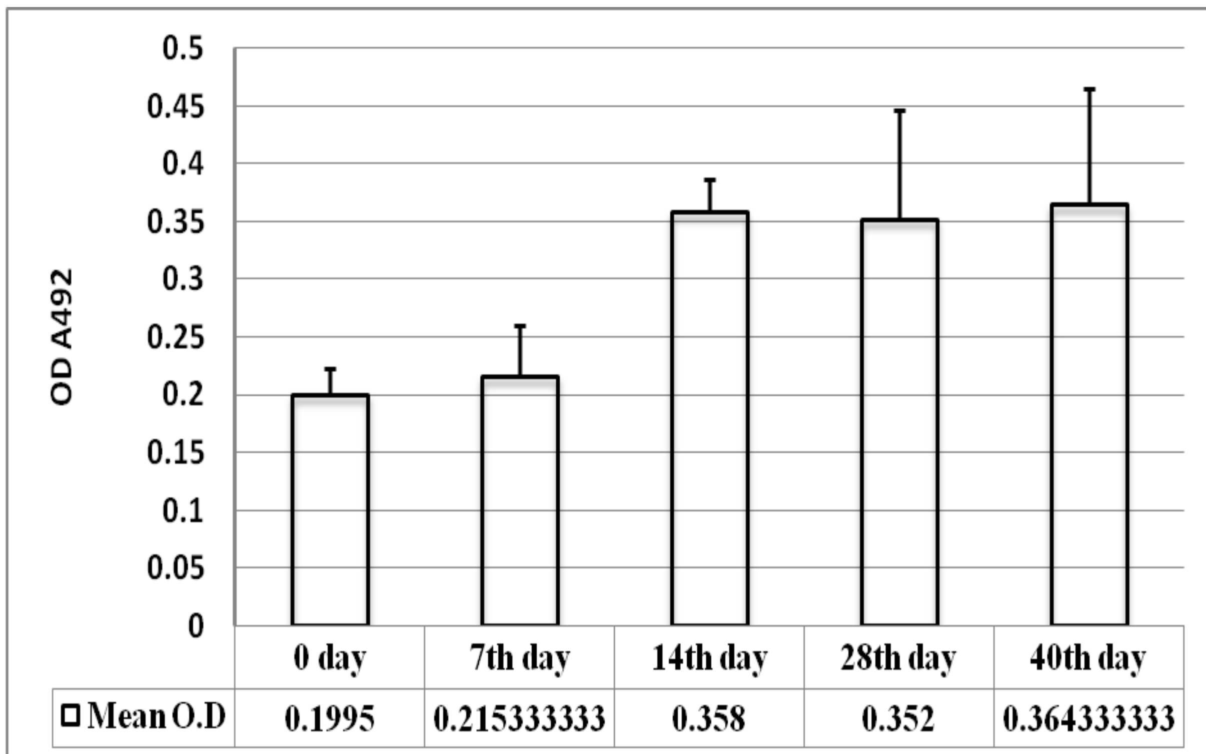
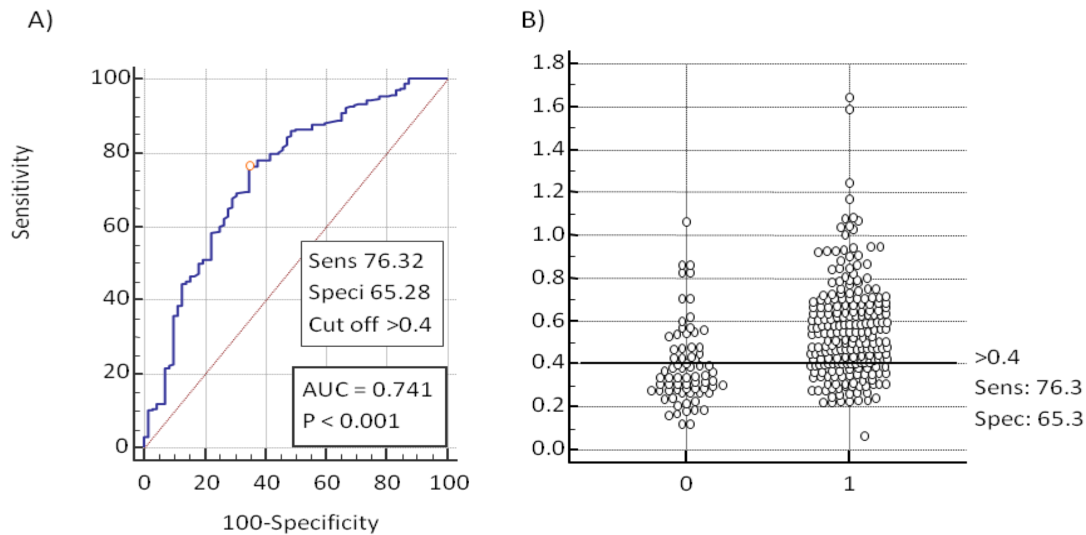


Fig.2 Time course analysis of the antibody production kinetics against C protein of PPRV in six vaccinated goats



	0 day	7th day	14th day	28th day	40th day
Mean O.D	0.1995	0.215333333	0.358	0.352	0.364333333

Fig.3 The ROC curve (A) and interactive dot diagram (B) obtained from a panel of 300 random sera from the field. The indirect ELISA developed using recombinant C protein of PPRV has a sensitivity of 76.3% and specificity of 65.28% when comparing the standard c-ELISA



The anti-goat horseradish peroxidase conjugate (Sigma–Aldrich, USA) was used @ 1:5000 in blocking buffer. OPD substrate solution (OPD 1 mg/mL containing 4 µL 3% H_2O_2) was added and incubated at 37 °C for 15 min followed by addition of 50 µL of 1 M H_2SO_4 to stop the reaction. The absorbance values were measured at 492 nm in an ELISA reader (Tecan, Switzerland).

The PPRV lineage four is expanding its territory in various regions of Africa, Europe, and Asia (Banyard *et al.,* 2010; Parida *et al.,* 2015). In the wake of the global control and eradication efforts, there is a need to develop modern vaccines and diagnostics that can differentiate the vaccinated from the infected animals (DIVA). At present, two groups have successfully rescued the PPR vaccine virus (Hu *et al.,* 2012; Muniraju *et al.,* 2015). However, currently, there is no marker vaccine and diagnostic test available in the market. Developing marker vaccine by altering the non-structural proteins can be an attractive option. To adopt this strategy, we have earlier reported the successful expression and development of a monospecific antibody against the non-structural C protein of PPRV from vaccine virus (Bhadouriya *et al.,* in press). In continuation of that, here we are reporting the reactivity profile of this protein against both the vaccinated and convalescent sera and we have also evaluated the antibody production kinetics of this protein in vaccinated goats.

The in-house optimized I-ELISA was used for determining the reactive pattern of convalescent and vaccinated sera. The optimum antigen (1µg per well) and antibodies (the goat sera @ 1:4) concentration to use in I-ELISA were determined earlier (Bhadouriya *et al.,* in press). The negative cut-off OD was determined to be 0.37 (mean + 2SD) using known negative sera (n=20) with an SNT titre of <1:2. C protein being non-structural, the reactivity pattern of convalescent (n=20) and vaccinated (n=20) sera may differ as the vaccine virus could multiply only a few rounds in the animal whereas in natural infection the virulent virus evades the host defense and could multiply

and spread to various tissues. However, we could find only minimal difference between the vaccinated and convalescent sera (Fig. 1). To get a conclusive picture, more sera from vaccinated and convalescent animals should be tested.

The time course analysis of antibody production kinetics reveals the seroconversion against this protein occurs from 14th-day post vaccination and the same level was maintained up to the study period of the 40th day (Fig. 2). This is not surprising as the live attenuated vaccine is expected to induce antibody against both structural as well as non-structural proteins. We have also evaluated the diagnostic potential of this protein using random field sera.

A total of three hundred field sera were tested both in c-ELISA and indirect ELISA and the result indicate that recombinant protein-based indirect ELISA has a sensitivity of 76.3% and specificity of 65.3% when compared with the standard c-ELISA (Fig. 3 A and B). The low level of sensitivity and specificity is expected as this protein is non-structural and short-lived in the host. More samples should be screened to get conclusive results.

In conclusion, here we have evaluated the reactivity pattern and antibody kinetics of the recombinant C protein of PPRV and the data generated in this study will be useful in developing marker vaccine and DIVA ELISA for PPR.

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Conflict of Interest

The authors declare no conflict of interest.

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