

Fig.1: (a) Antibody against BoHV-1 elicited by vaccines in Mice. Mice were intradermic immunized twice at a 20 days interval. Arrow indicates the date at with booster vaccination was performed. The cut-off was calculated as the mean level of antibody at 0 days post vaccination (dpv) + 2 SD. The results are presented as the mean \pm standard error of the mean (SEM) of log₁₀ antibody titers (n=5). Significant difference *(p<0.05), ** (p<0.01), *** (p<0.001) compared to pCIgD group. **(b)** Anti-BoHV-1 specific immunoglobulin isotypes measured by direct ELISA at 40 dpv. Sera was diluted at 1/50. Each bar represented mice serum OD A492 + SEM of the groups. (n=5) Significant differences *(p<0.05), ** (p<0.01), *** (p<0.001) compared with pCIgD group.

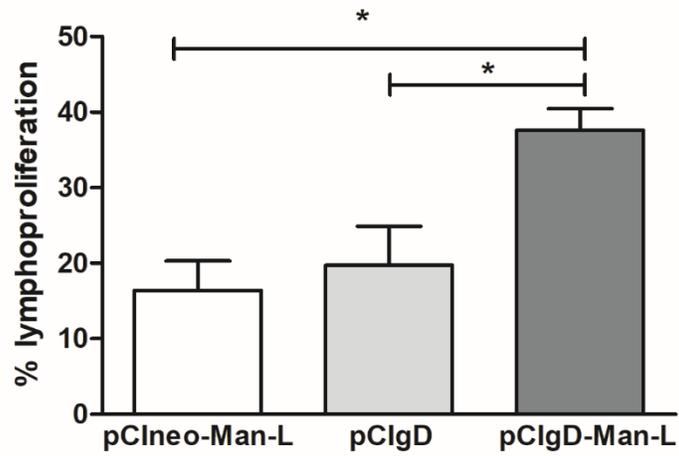
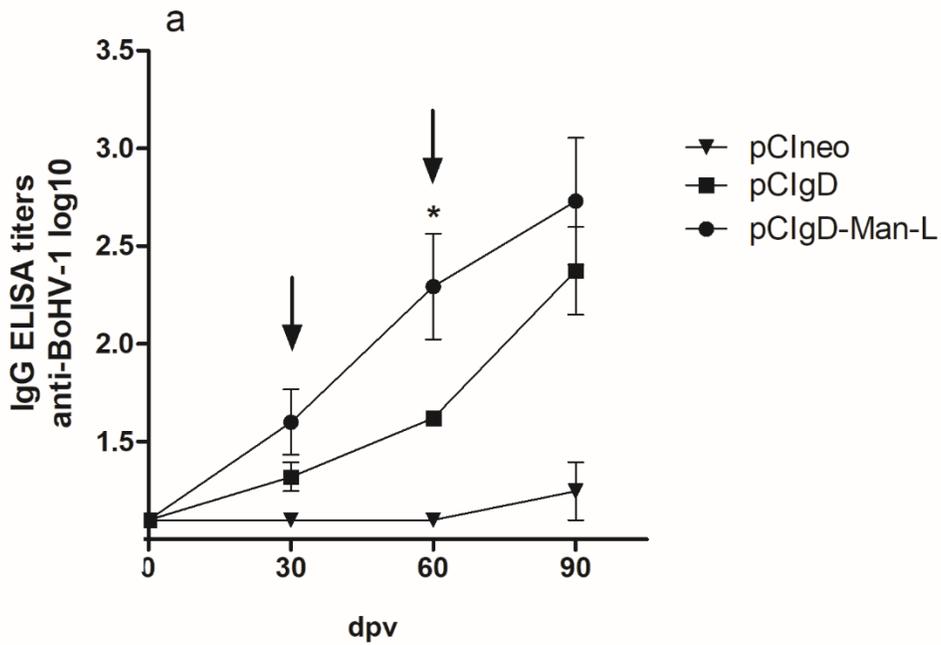


Fig.2: Specific proliferation of splenocytes from vaccinated mice measured by CFSE labeling. Percentages of proliferating cells were evaluated by cytometry at 120 dpv. Cells were stimulated *in vitro* with iBoHV-1. The bars represented the mean of positive CFSE cells \pm SEM n=5. Significant differences respect pCIgD and pCIneo-Man-L group *(p<0.05).



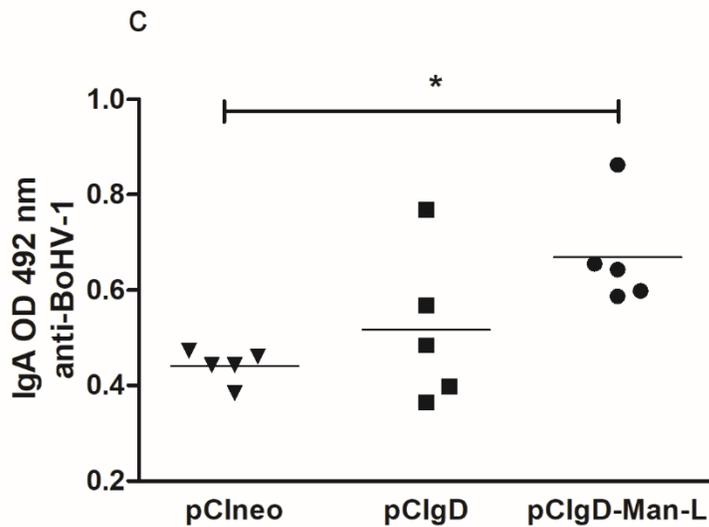
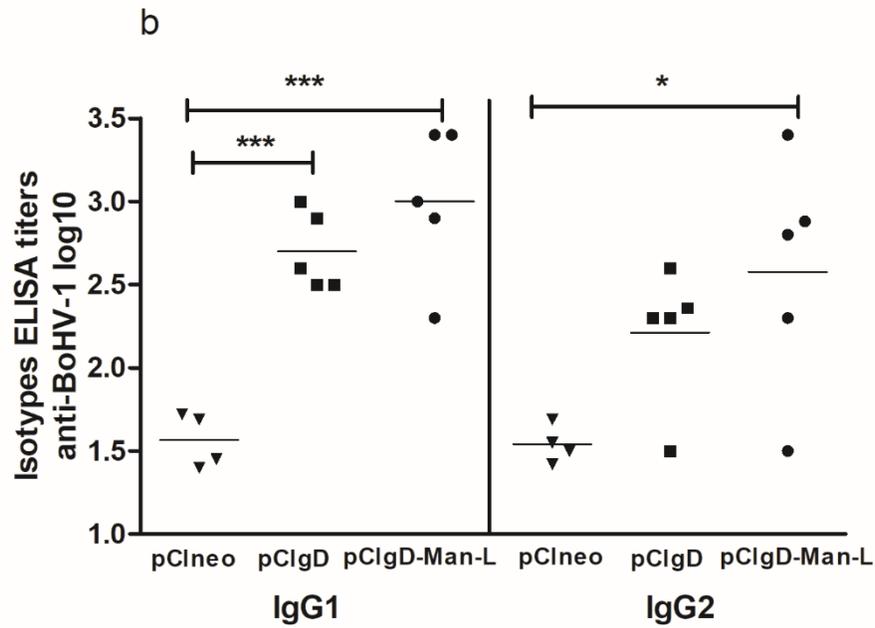


Fig.3: (a) Humoral immune response in bovine. Animals were immunized three times at 30 days intervals. Arrow indicates the date at with booster vaccination was performed. The cut-off was calculated as the mean level of antibody at 0 days post vaccination (dpv) + 2 SD. The results are presented as the mean \pm SEM of log₁₀ antibody titers (n=5) *(p<0.05) vs pCIgD group. (b) Anti-BoHV-1 specific immunoglobulin isotypes in sera measured by indirect ELISA at 90 dpv. The results are presented as the mean \pm SEM of log₁₀ antibody titers (n=5). *(p<0.05), *** (p<0.001) vs pCIneo group. (c) Anti-BoHV-1 specific immunoglobulin IgA isotypes in nasal swabs measured by indirect ELISA at 6 dpc. The results are presented as the mean \pm SEM of the O.D (n=5). *(p<0.05) vs pCIneo group.

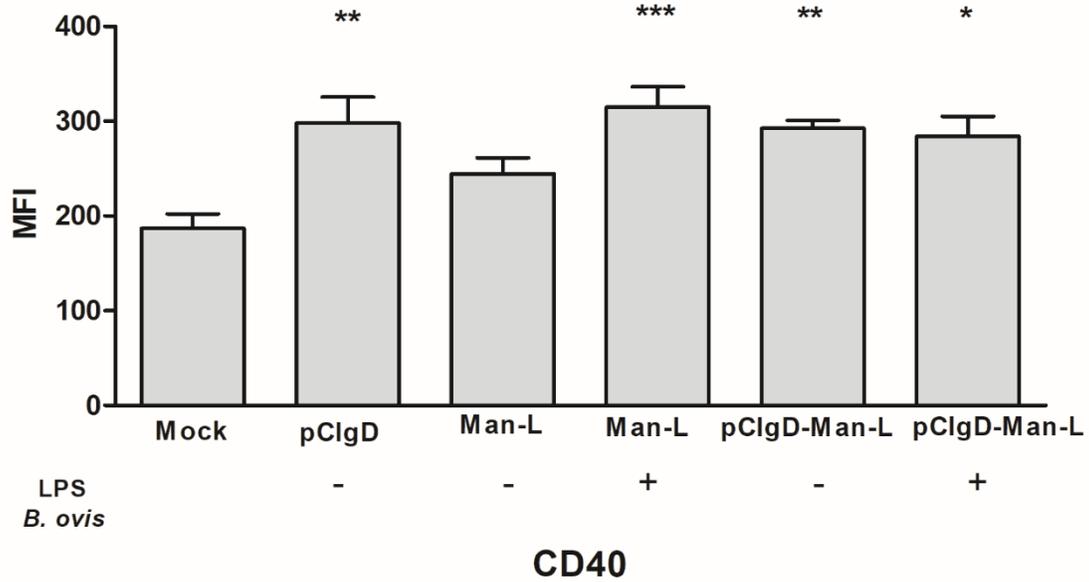


Fig.4: Cellular response elicited. Activation of ALDCs after 16 h incubation with culture medium (mock), pCIgD, Man-L or pCIgD-Man-L with (+) or without (-) LPS. Each bar represents the mean fluorescence index for three replicates plus SEM. *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$) vs mock.

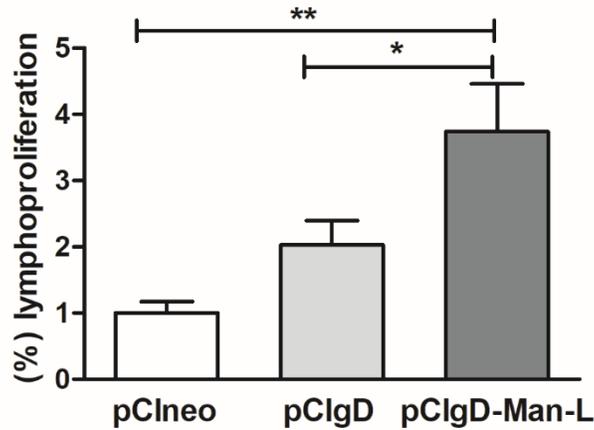


Fig.5: Percentages of proliferating cells at 12dpc. Cells were stimulated *in vitro* with iBoHV-1. The bars represent the mean of positive CFSE cells \pm SEM. ** ($p < 0.01$) vs control group, * ($p < 0.05$) vs pCIgD group.

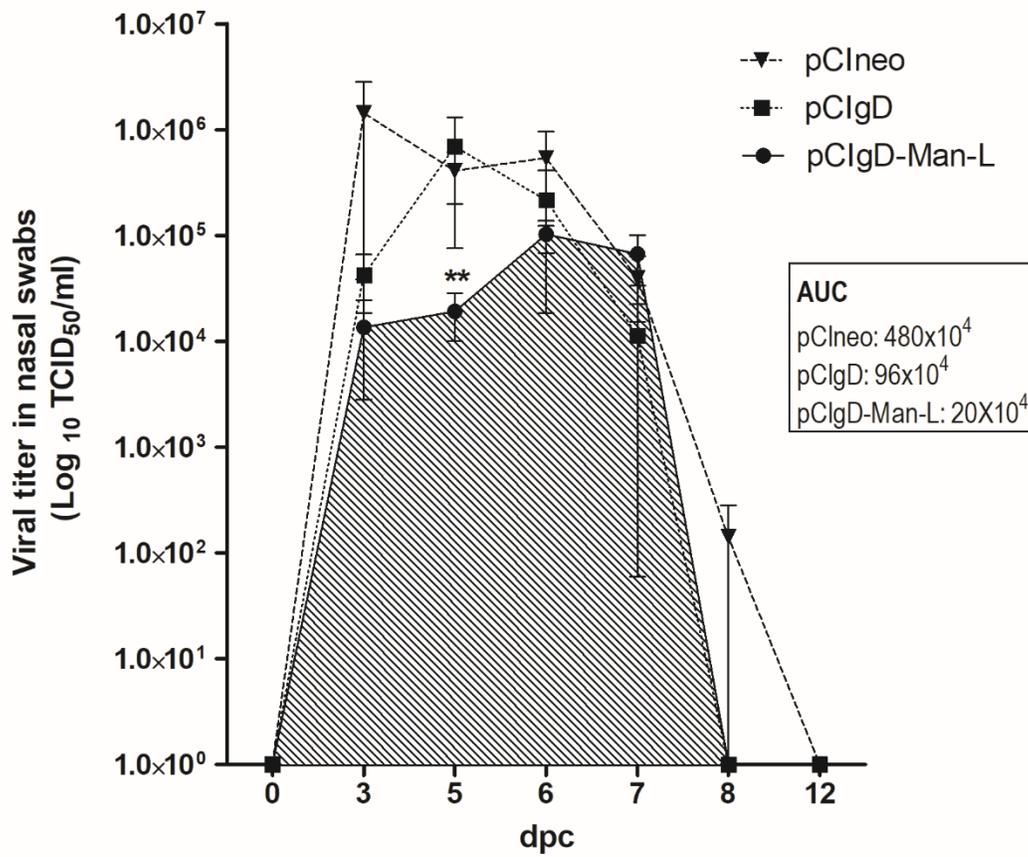


Fig. 6: Viral excretion after challenge. At 90 dpv the animals were challenged by aerosol exposition with BoHV-1. Nasal swabs were obtained at 0, 3, 5, 6, 7, 8 and 12 days post challenge. Viral titres in nasal swabs were expressed as log₁₀. ** (p<0.01 vs pCIneo group). AUC: area under curve.

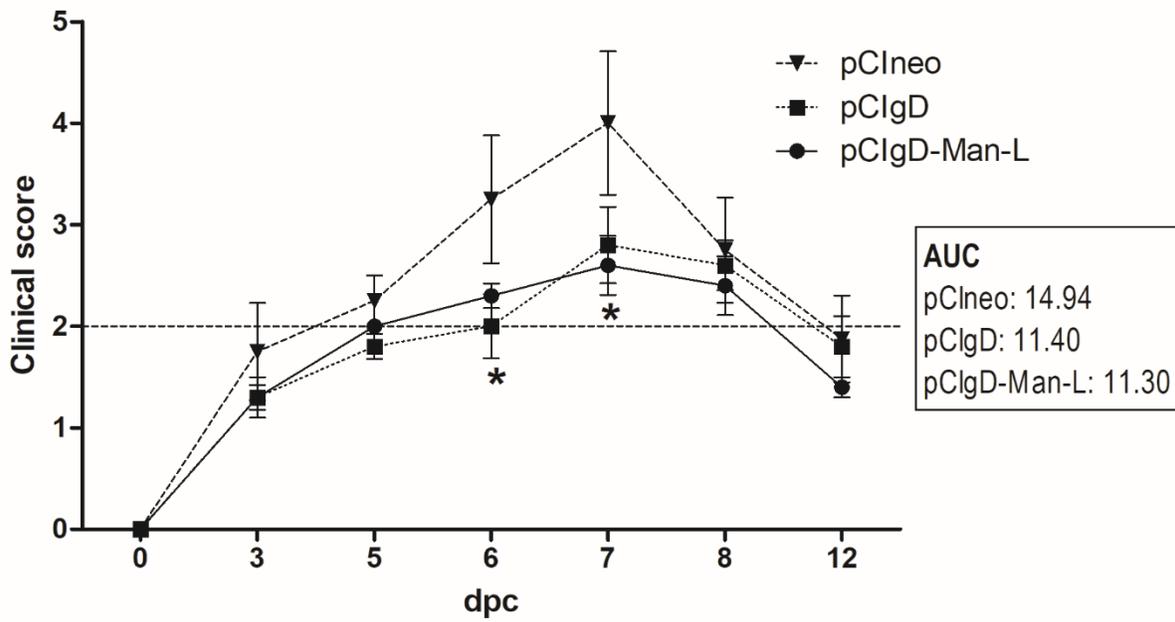


Fig.7: Clinical score after viral challenge. A clinical score was established according to the presence and severity of mucus and conjunctivitis. Dotted line represents a clinical score of 2, which was considered as mild sickness. Score was expressed as mean number of clinical score \pm SEM of each group in each time point. *($p < 0.05$) vs pCIneo group. AUC: Area Under Curve.