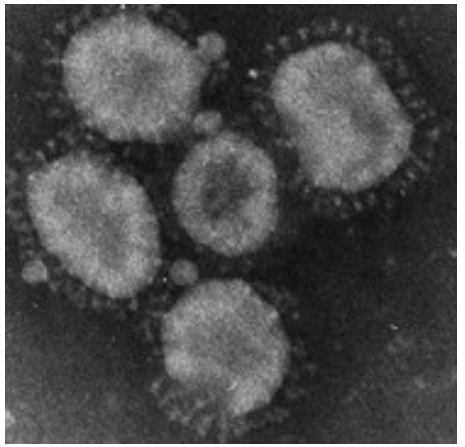
Self-adjuvanted nanoparticle-based vaccines for avian infectious bronchitis

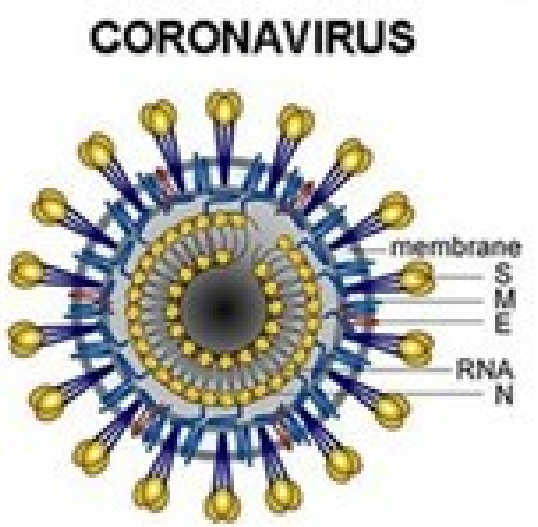
- Infectious bronchitis (IB)
- Challenges of current IB vaccines
- SAPN vaccine as an alternative
- Results-IB project
- Conclusions

Infectious bronchitis (IB)

- Clinical signs: respiratory signs, ocular discharge, weight loss, deformed egg, nephritis in some cases.



- Etiology: infectious bronchitis virus (IBV), the gamma *Coronaviridae,* ~80-200 nm, lipid envelope, single stranded, positive RNA virus, consist of 4 structural protein (Spike, Membrane, Envelope and Nucleoprotein).



(Veterinary Record, 1983, 112, 104-105) (Abu-Median P. pp.125-129. Rauischholzhausen, Germany, 2009)

Challenges in controlling IB

- Large numbers of IBV variants attributed by point mutation and recombination.
- Current vaccines (live attenuated): lack of cross-protection and contribute to emerging new variant strains
- Maternal antibodies: limit live vaccines in young chicks

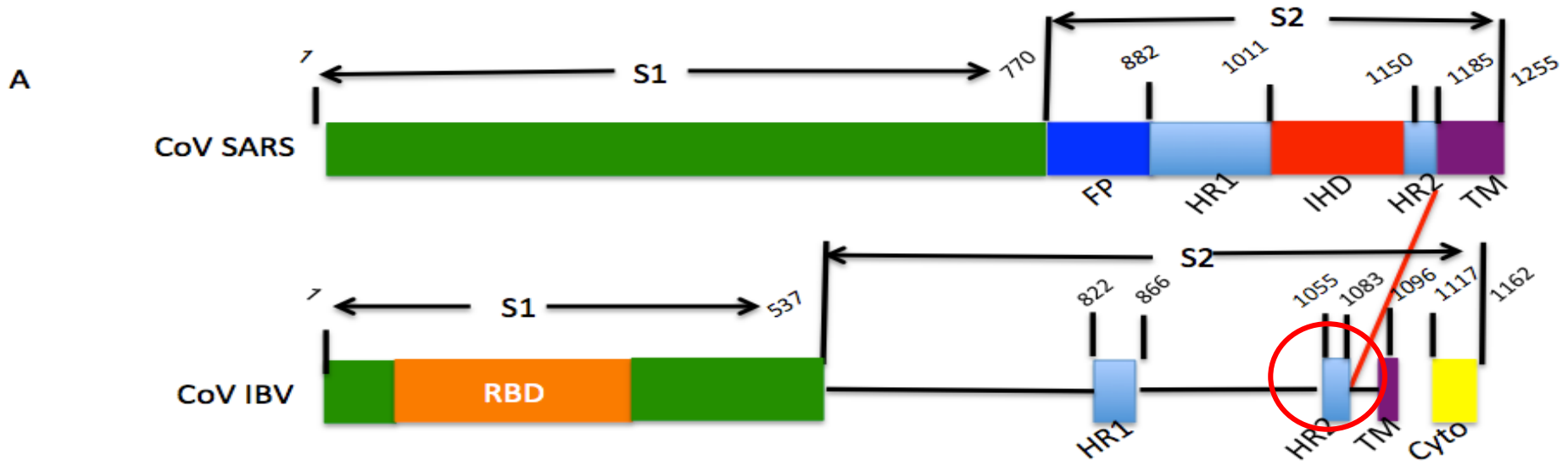
objective

Self-assembled protein nanoparticle formulations are able to preserve and present conformational conserve epitopes and further protect against IBV infections in chickens.

Further

- Generate a self-adjuvanted vaccine prototype presenting trimeric epitopes derived from the spike protein of IBV, to induce protection against IBV infections in chickens.

IBV target epitopes

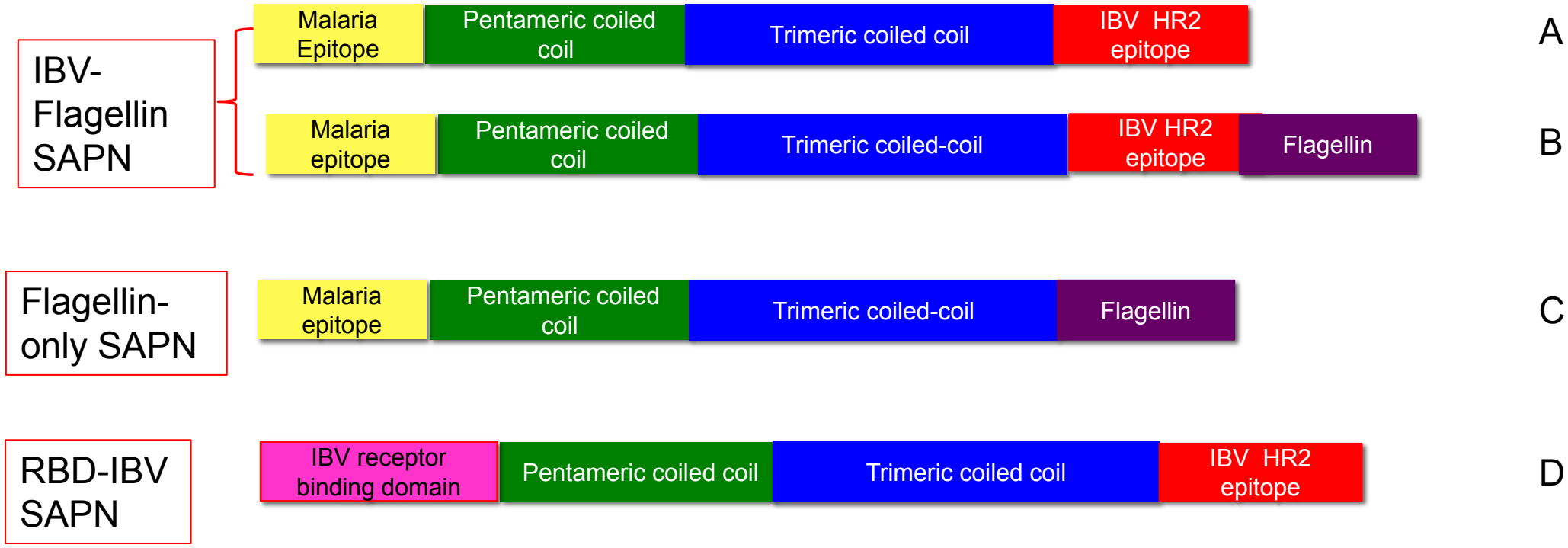


B

Sequence comparison of HR2 coiled coil

```
                    a   d   a   d   a   d   a   d   a   d
CoV IBV-M2188 ILDIDSEIDRIQGVIQGLNDSLIDLEKLSILKTYIKWPWYVWL
CoV SARS      VVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWL
                *   ****   *   **  *****  *     **********
```

IBV nanoparticle nomenclatures and their composition

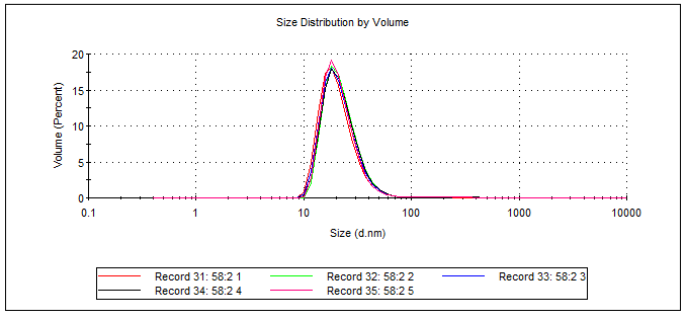


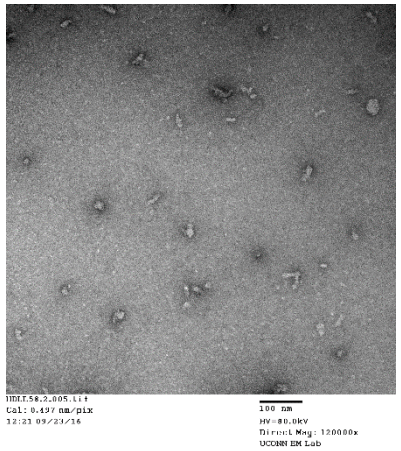
Results:

DLS and TEM characterization of SAPNs for IBV

A

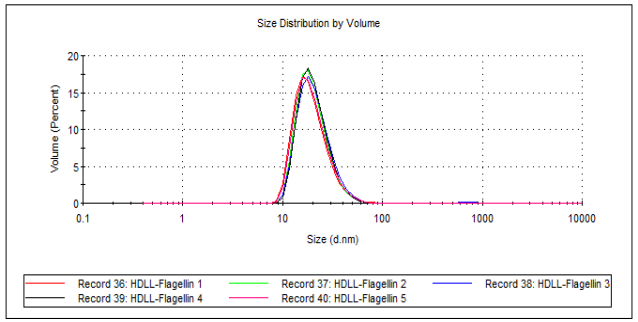
		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 45.77	Peak 1:	22.76	100.0	20.61
PdI: 0.426	Peak 2:	0.000	0.0	0.000
Intercept: 0.953	Peak 3:	0.000	0.0	0.000
Result quality : Good				

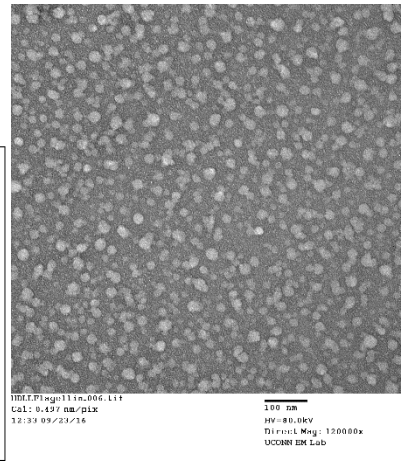




B

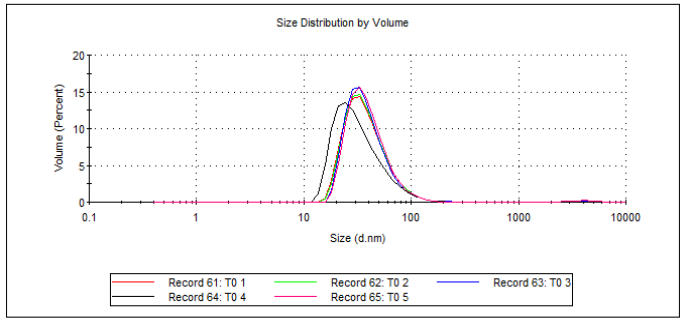
		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 32.83	Peak 1:	20.02	99.7	8.570
PdI: 0.335	Peak 2:	532.9	0.3	278.0
Intercept: 0.950	Peak 3:	0.000	0.0	0.000
Result quality : Good				

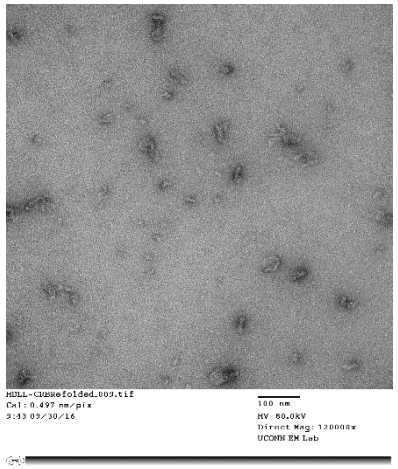




C

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 60.16	Peak 1:	40.36	99.0	21.12
PdI: 0.244	Peak 2:	4223	1.0	1035
Intercept: 0.974	Peak 3:	0.000	0.0	0.000
Result quality : Good				



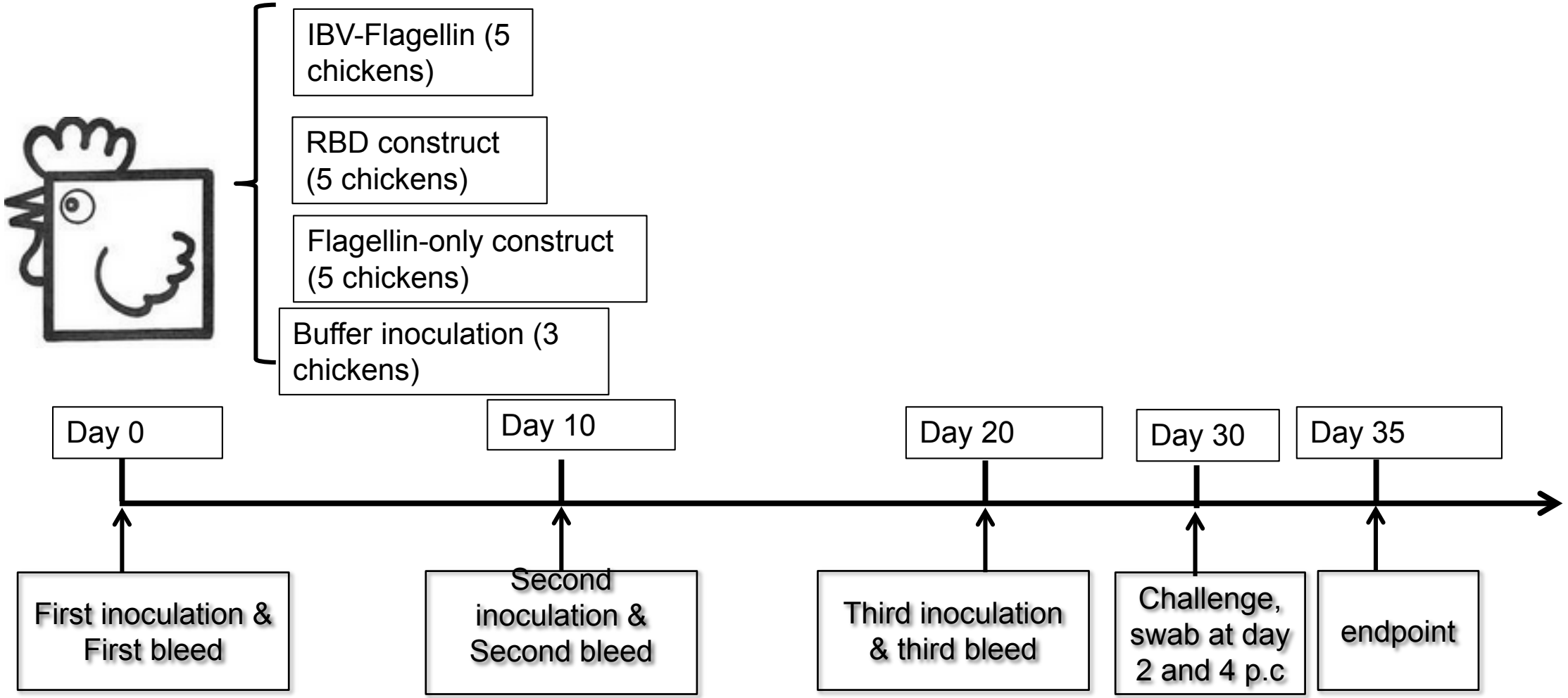


A: IBV-Flagellin SAPN

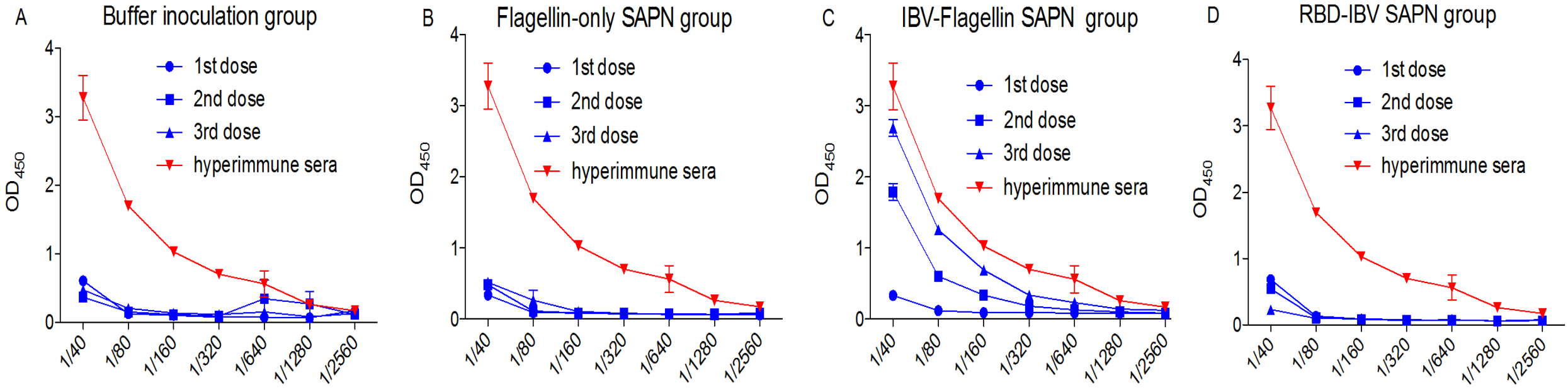
B: Flagellin-only-SAPN

C: RBD-IBV SAPN

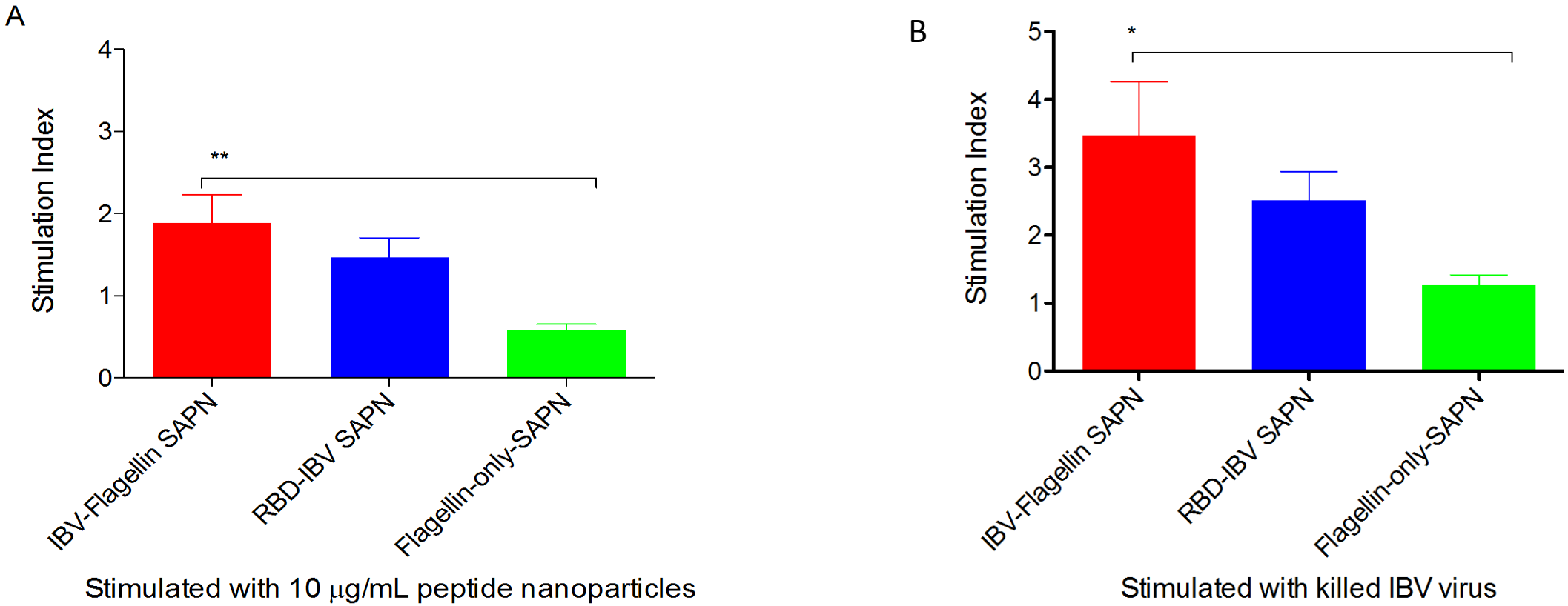
Testing vaccine immunogenicity and efficacy



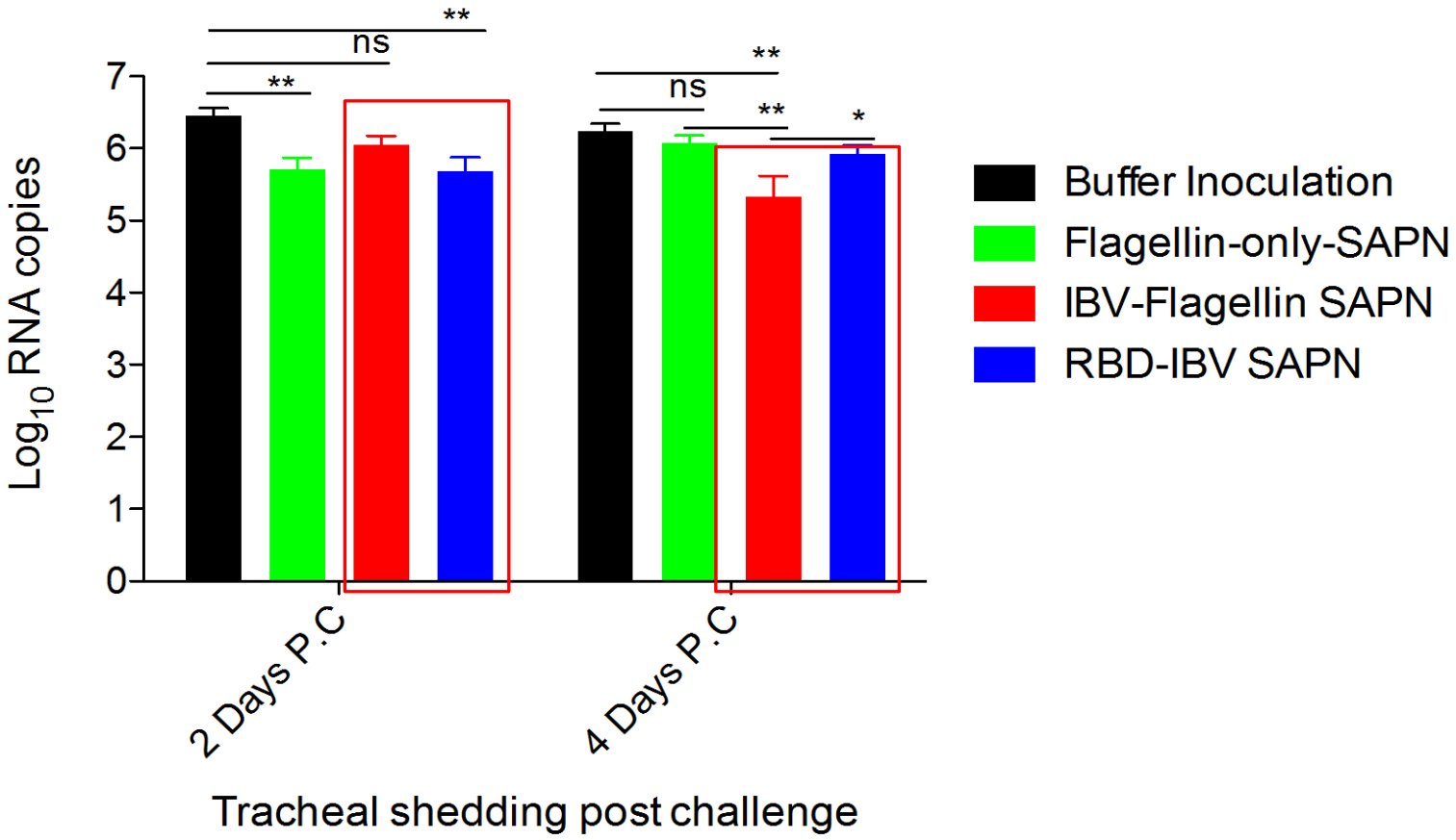
ELISA


A
Buffer inoculation group
B
Flagellin-only SAPN group
C
IBV-Flagellin SAPN group
D
RBD-IBV SAPN group
1st dose
2nd dose
3rd dose
hyperimmune sera
OD450
0
1
2
3
4
1/40
1/80
1/160
1/320
1/640
1/1280
1/2560

Lymphocyte proliferation

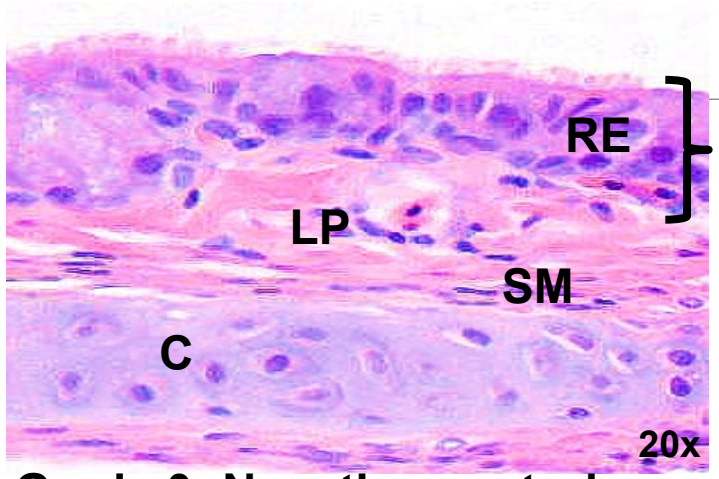


Tracheal shedding



Histopathology

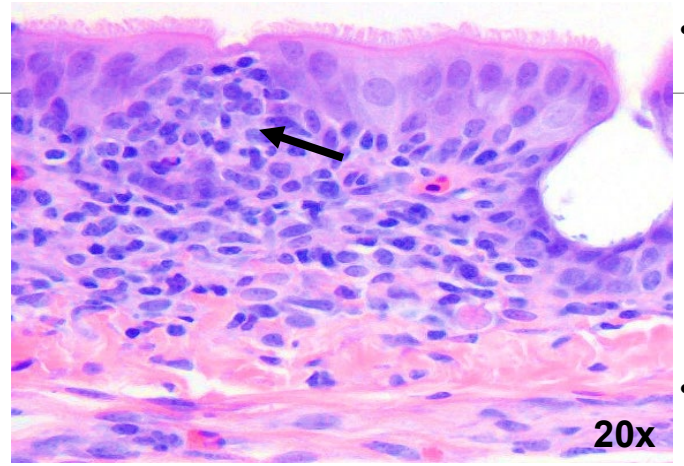
A



pseudostratified columnar cells with cilia

Grade 0: Negative control

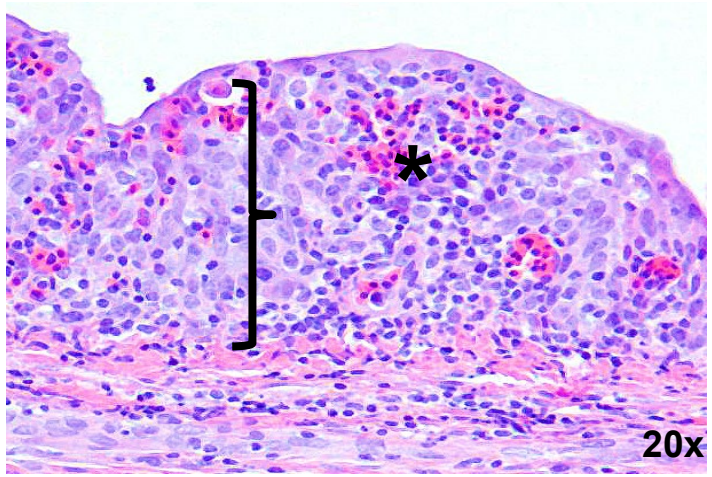
B



- infiltration of lymphocytes, macrophages and few heterophils transmigrating and infiltrating lamina propria and submucosa
- developing germinal centers observed at this grade

Grade 1: Mild

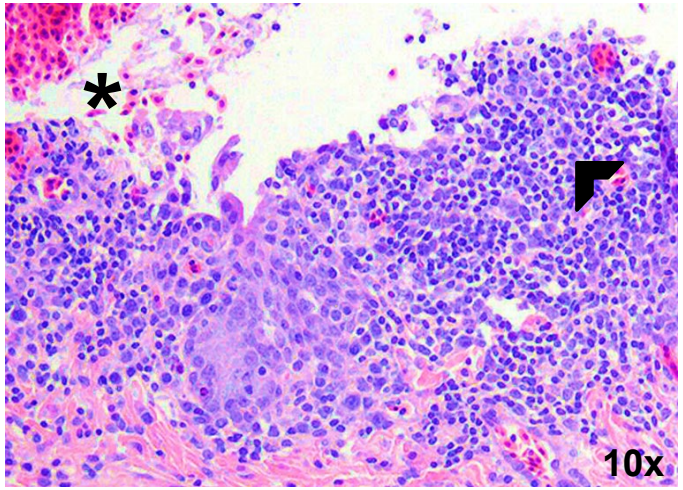
C



- desquamation of ciliated cells
- marked epithelial hyperplasia
- infiltration of lymphocytes, macrophages and heterophils (mucosa and submucosa)
- congestion and hemorrhage.

Grade 2: Moderate

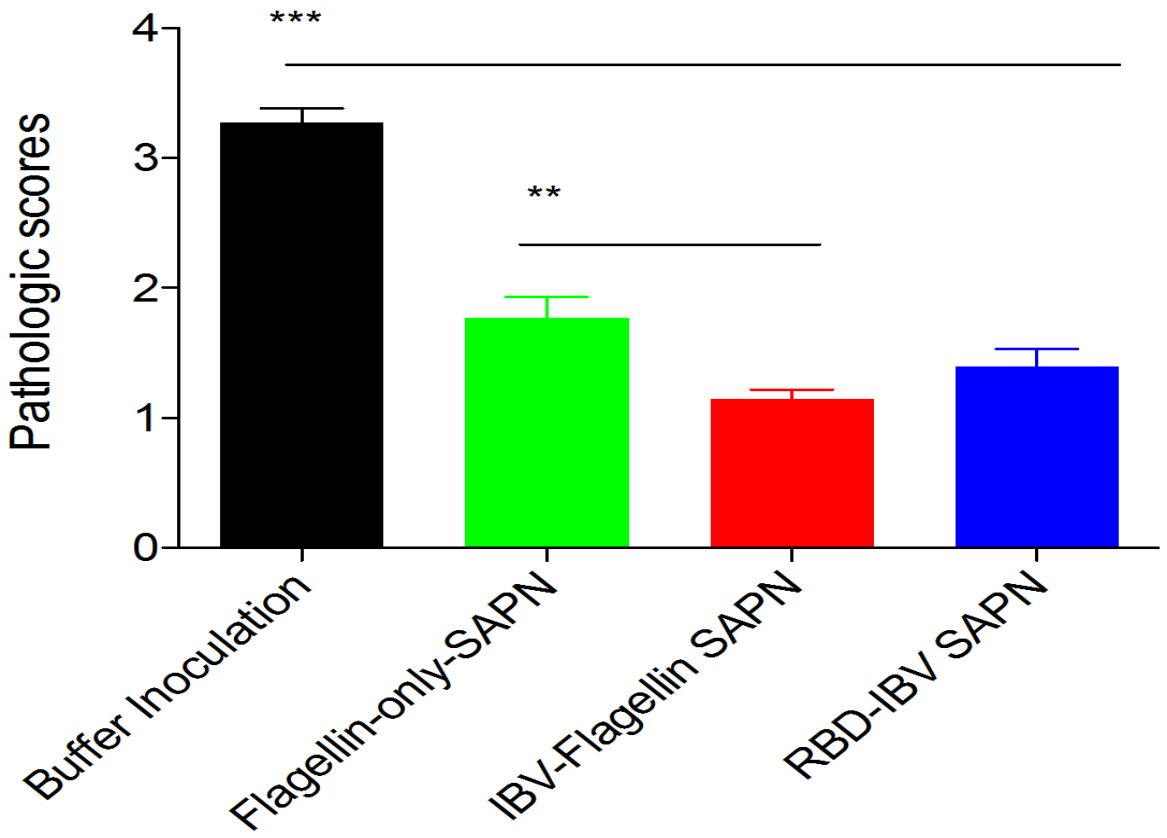
D



- large number of infiltrates (mucosa and submucosa)
- mucosa completely obliterated
- Fibrinous exudate in lumen admixed with RBC and necrotic debris
- congestion and hemorrhage

Grade 3: Severe

Histopathologic scores



Summary

- Chickens vaccinated with IBV-Flagellin elicited moderate level of antibody responses.

- PBMCs from chickens vaccinated with IBV-Flagellin or RBD-IBV SAPN had significantly higher level of lymphocyte proliferation stimulated by either the nanoparticles or the inactivated IBV virus

- Chickens vaccinated with IBV-Flagellin or RBD-IBV SAPN had significantly less histopathologic scores in tracheal tissues than no vaccine group, indicating protection conferred by the vaccines

Conclusions

- SA-MC-Penn vaccine prototype provided partial protection to vaccinated chickens against a challenge with a HPAIV H5N2.

- The addition of adjuvant MPLA improved the immunogenicity of SA-MC-Penn against challenge with a LPAI H5N2 in vaccinated chickens

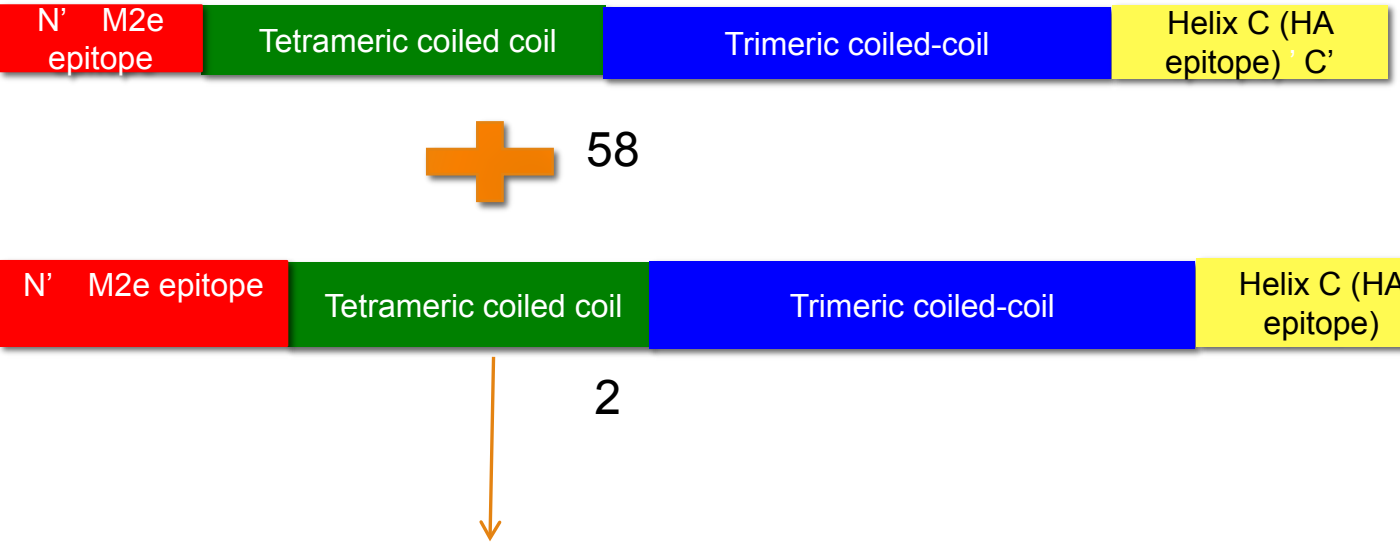
- IBV vaccine prototypes IBV-Flagellin or RBD-IBV SAPN provided protection against challenge with a IBV M41 strain in vaccinated chickens.

Acknowledgements

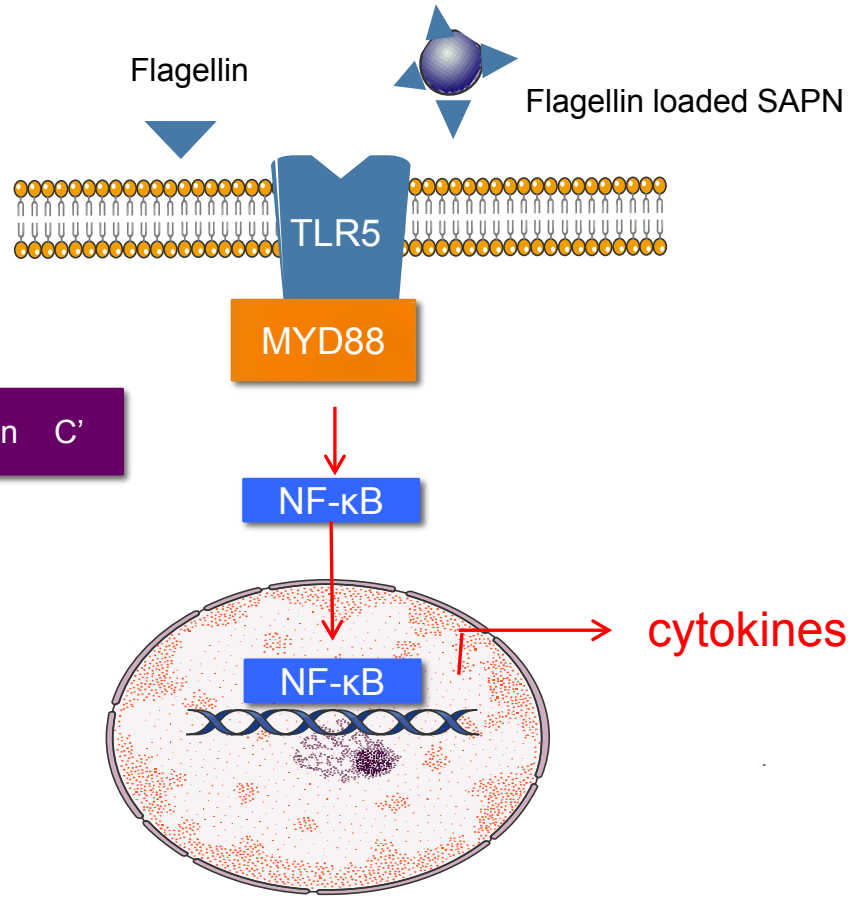
This research is funded by USDA-NIFA PRDCAP-Project

Thank you

A self-adjuvanted SAPN: incorporate flagellin molecule

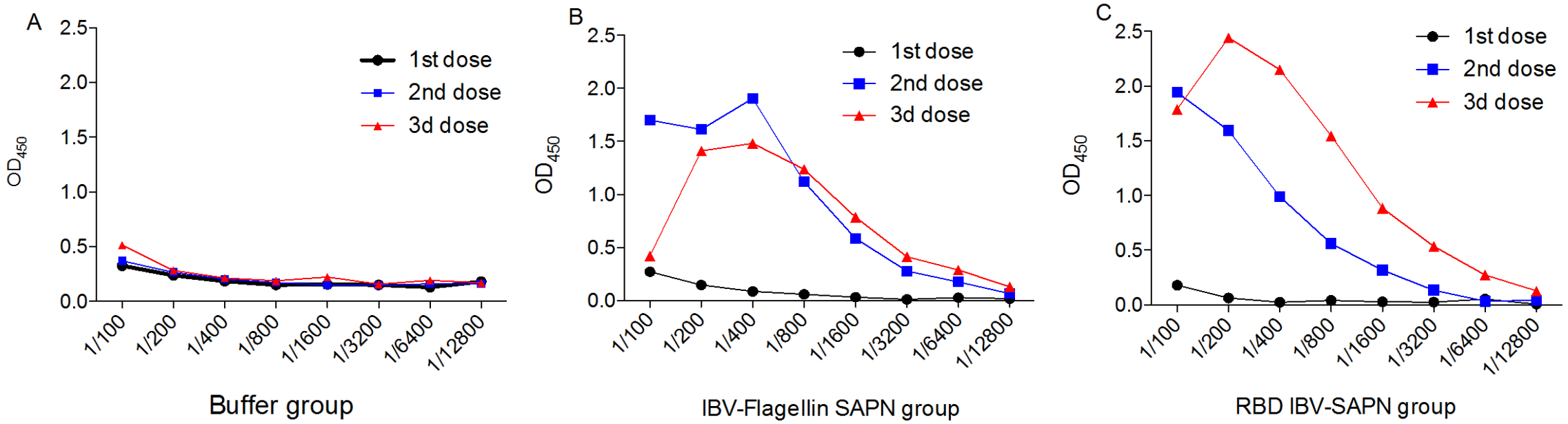


Co-assembled at molar ratio of 58: 2

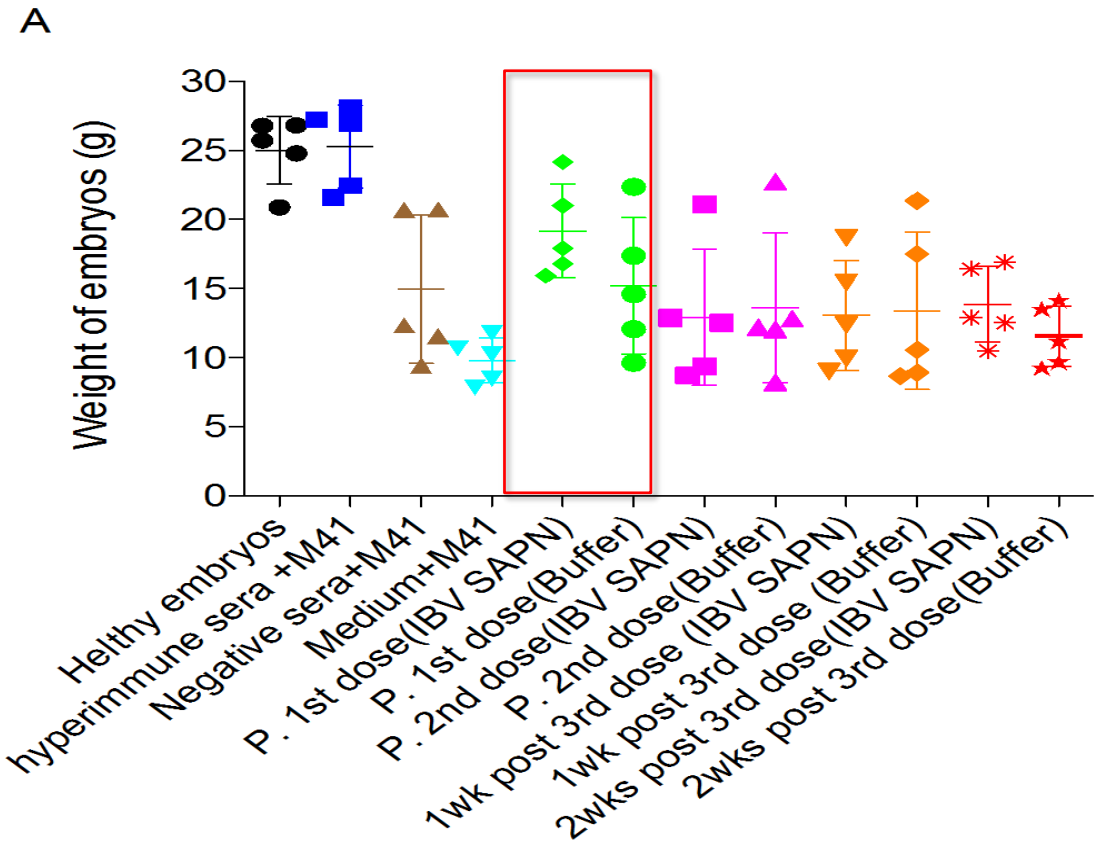


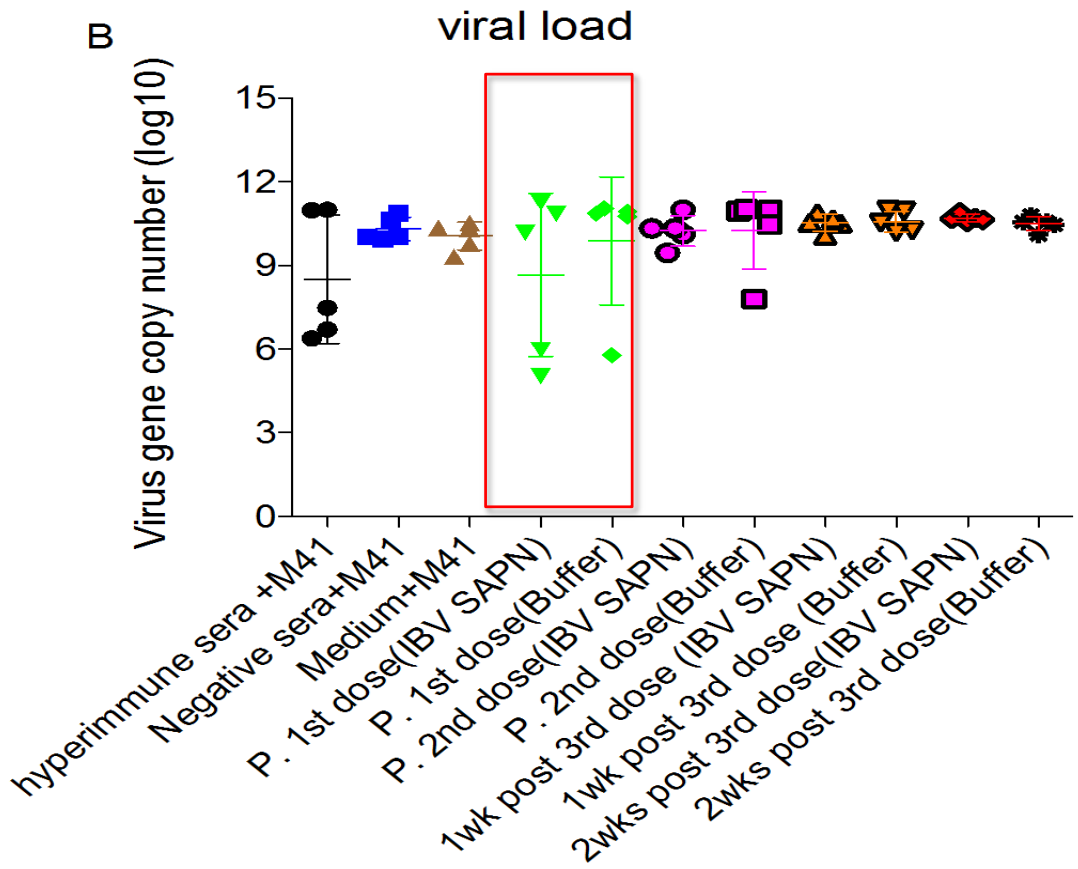
Chickens vaccinated with IBV-Flagellin or RBD-IBV SAPN elicited high level of antibody responses

Figure 18

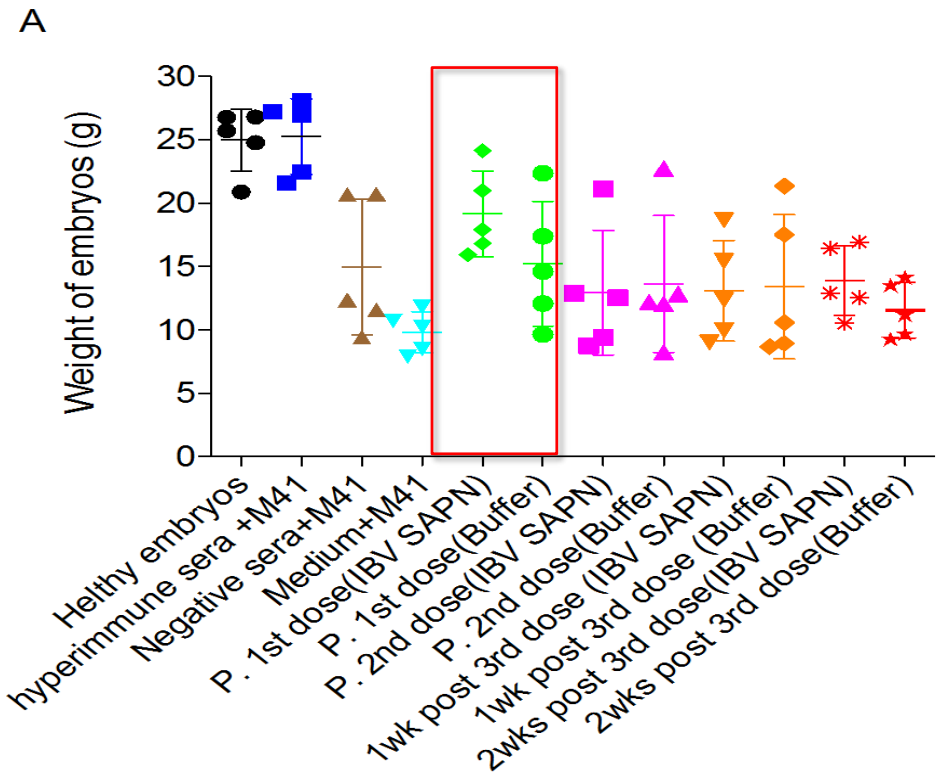


Antibodies induced by IBV SAPN neutralizing infection of IBV M41 in embryos





Antibodies induced by IBV SAPN neutralizing infection of IBV M41 in embryos



Healthy embros

Hyperimmune serum+M41

IBV SAPN immunized sera+M41 (2wks post 1 dose)

Buffer inoculated sera+M41 (2wks post 1 dose)

Medium+M41 (2wks post 1 dose)

Chickens vaccinated with IBV-Flagellin or RBD-IBV SAPN significantly reduced the shedding of infectious IBV viruses in tracheas

Figure 21