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PROGRESS REPORT PRD-CAP

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NEW PREVENTION AND CONTROL STRATEGIES FOR POULTRY RESPIRATORY DISEASES

PIs

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Protection Conferred by Infectious bronchitis
Virus Spike Ectodomain (S1 + S2 ectodomain)



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Vaccine

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Protection against infectious bronchitis virus by spike ectodomain subunit vaccine



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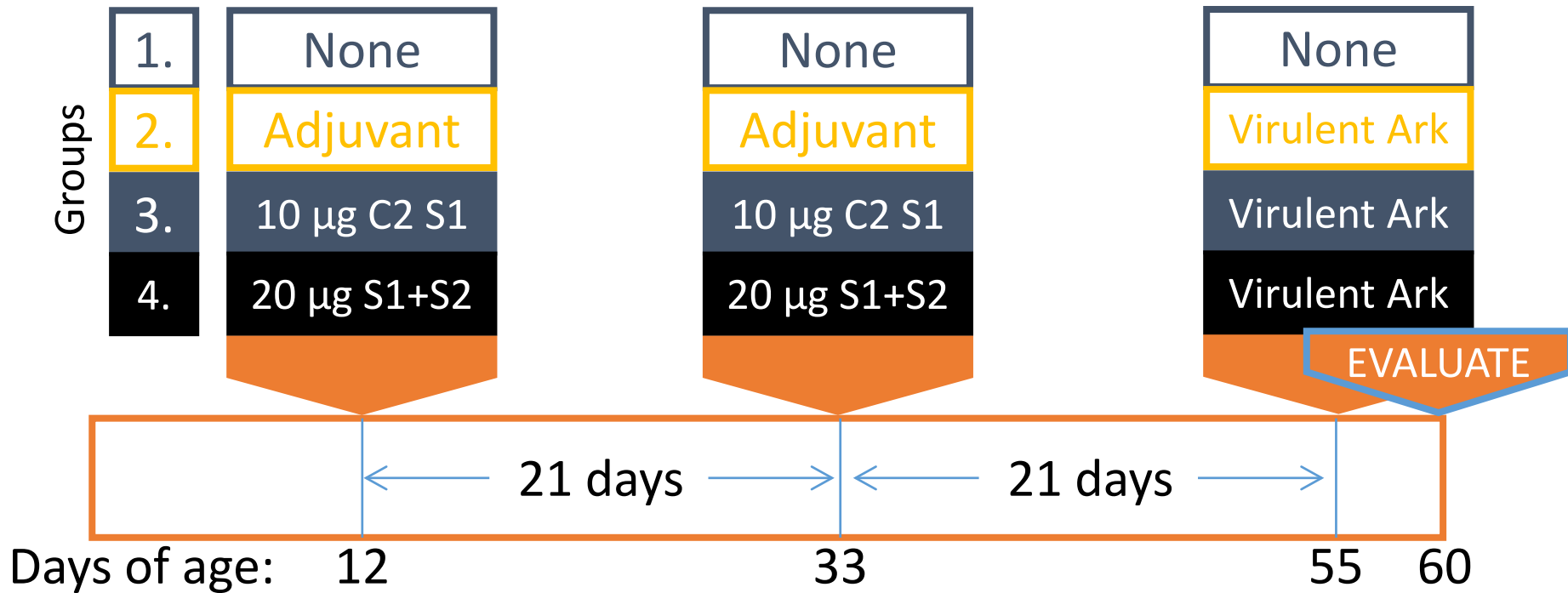
Acknowledgments

We thank Cassandra Breedlove Kitchens, Natalia Petrenko, Steven Gulley and Cynthia Hutchinson for excellent technical assistance, and Dr. M. Hélène Verheije (Utrecht University) and members of her lab for constructing the S1 expression vector and teaching us their recombinant spike protein production, purification, and tissue binding protocols. Fatma Eldemery was sponsored by a scholarship from the Egyptian government (Egyptian Cultural and Educational Bureau, Washington, DC). Research was funded by United States Department of Agriculture's PRD-CAP grant 2014-08054 and State of Alabama Animal Health and Disease Research.

Trimeric recombinant S1 and S1+S2 ectodomain proteins were expressed in 293T cells using synthetic genes representing a distinct IBV Ark-type vaccine subpopulation selected in chickens

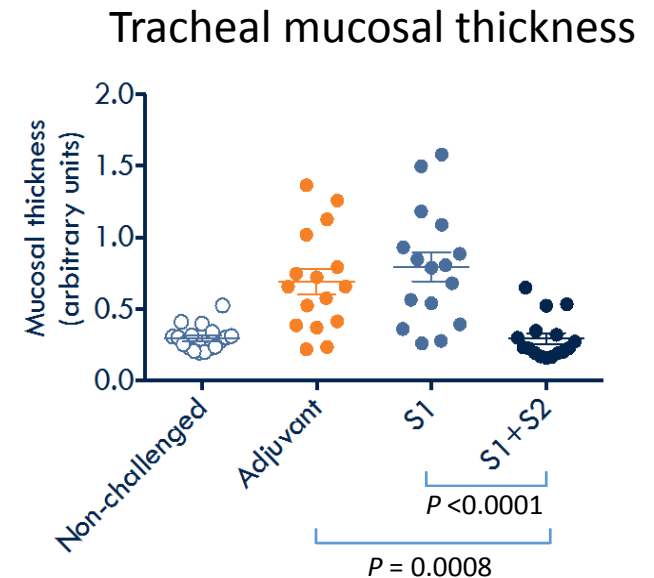
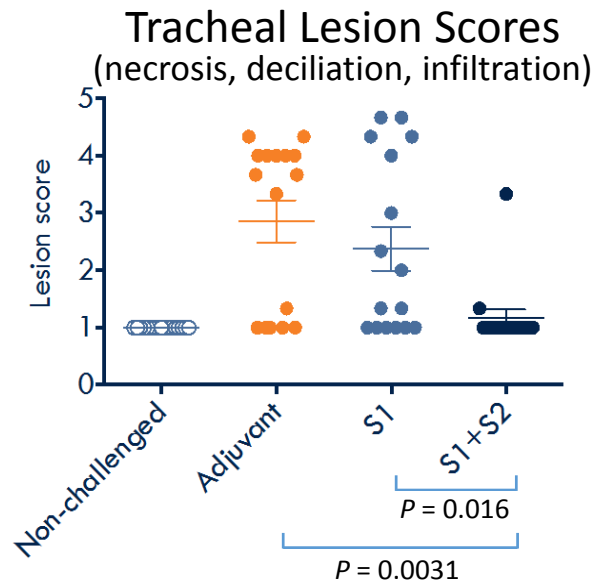
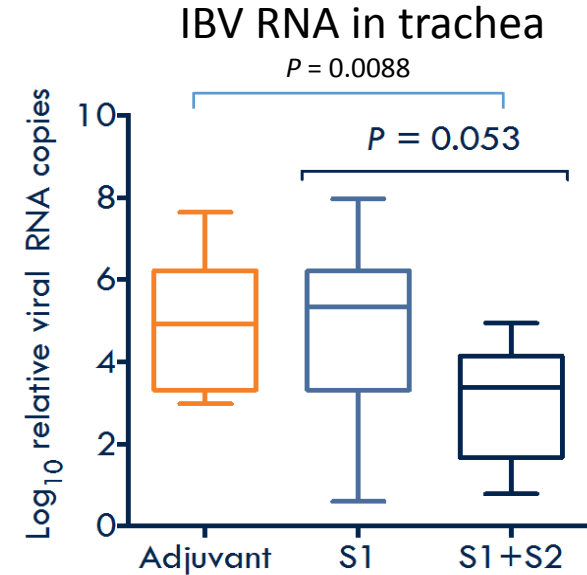
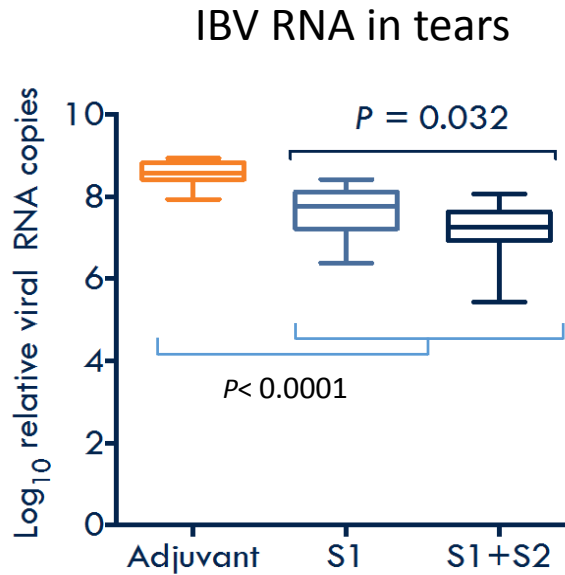
Chickens were immunized to determine the effect of the extension of the S1 domain with S2 ectodomain on protection against IBV.

Experimental design

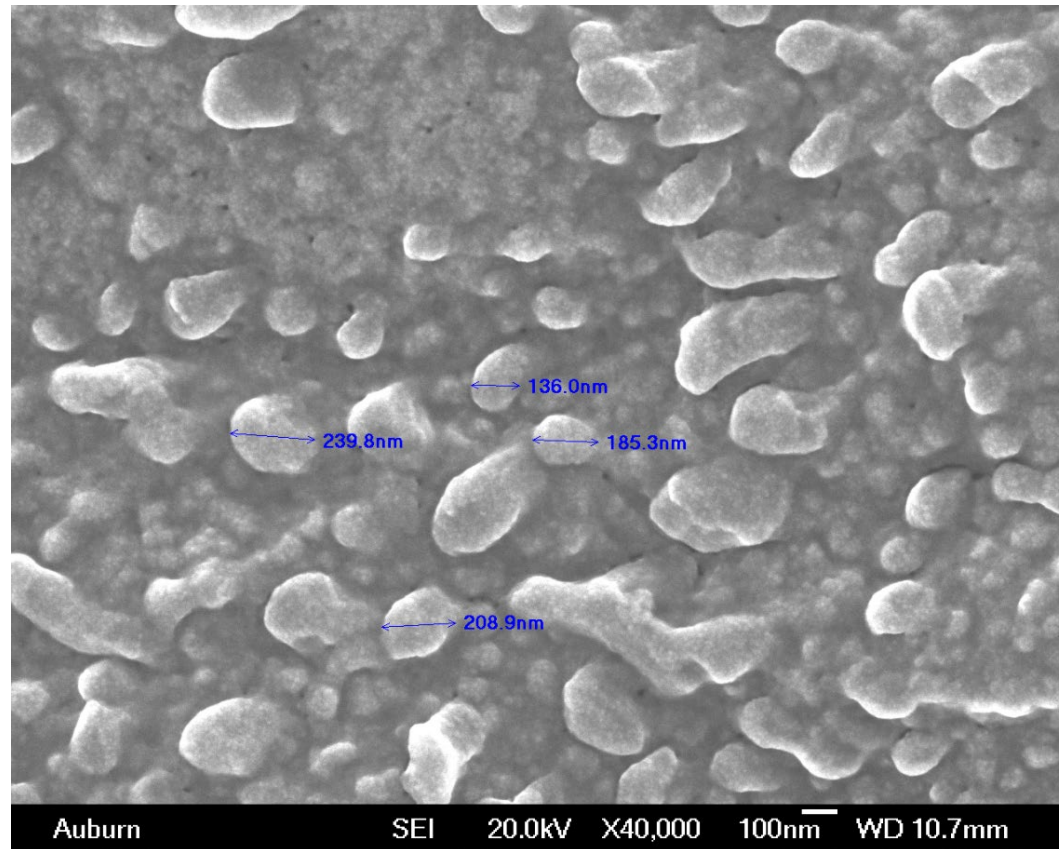


- 16 SPF white leghorn chickens/group
- Commercial mineral oil emulsion adjuvant MONTANIDE™ ISA 71 VG
- Ocular challenge: 10^5 EID₅₀ virulent Ark IBV

Effective protection by IBV S ectodomain recombinant protein

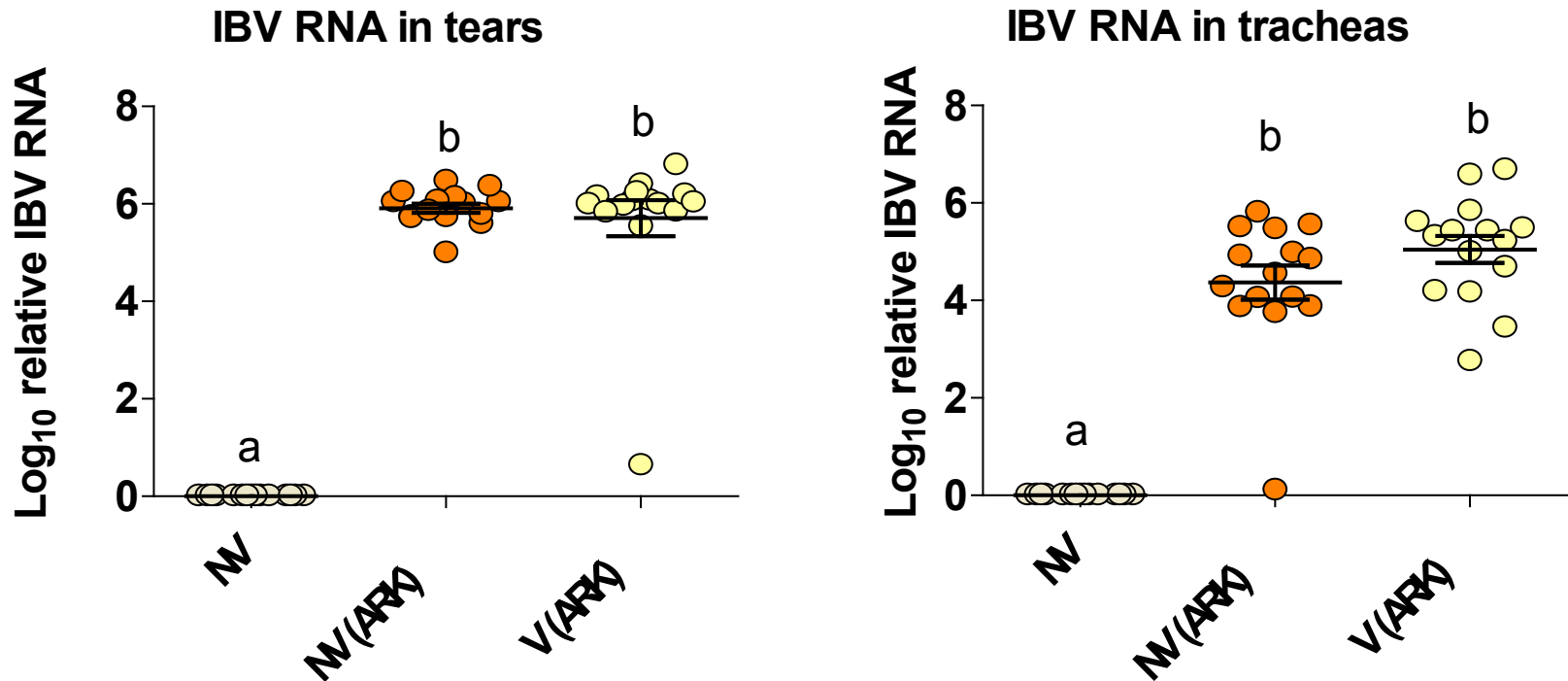


Recombinant IBV Spike Ectodomain Protein Encapsulated in Chitosan Nanoparticles



Scanning electron microscope of Chitosan-S protein nanoparticles

Viral load (IBV RNA) in tears and trachea of chickens vaccinated via nostrils at 7 days-old with Chitosan- S protein nanoparticles and challenged at 28 days old with virulent Ark IBV



IBV RNA determined 5 days post-challenge by qRT-PCR (5UTR gene).

In 2018

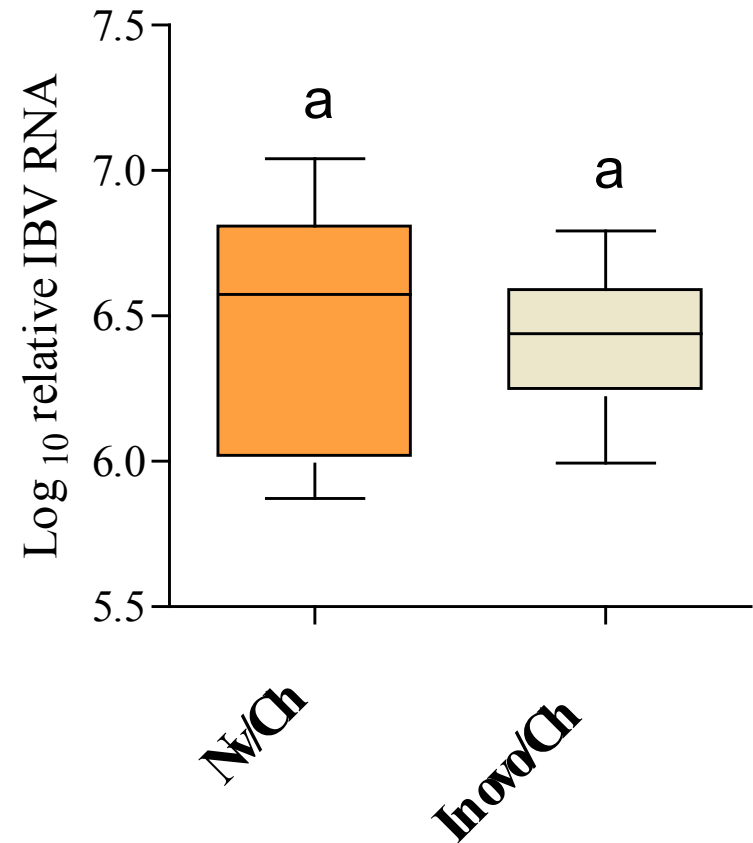
In ovo Vaccination with Recombinant IBV S Ectodomain
Protein Encapsulated in Chitosan Nanoparticles

Viral load (IBV RNA) in tears of chickens vaccinated *in ovo* with Chitosan- S protein nanoparticles and challenged at 21 days old with virulent Ark IBV

	Groups		
	Nv	Nv/Ch	V/Ch
In ovo D 18	-	Chitosan	Chitosan/ S ectodomain
Challen. D 21	-	Ark	Ark

n=12 chickens/group

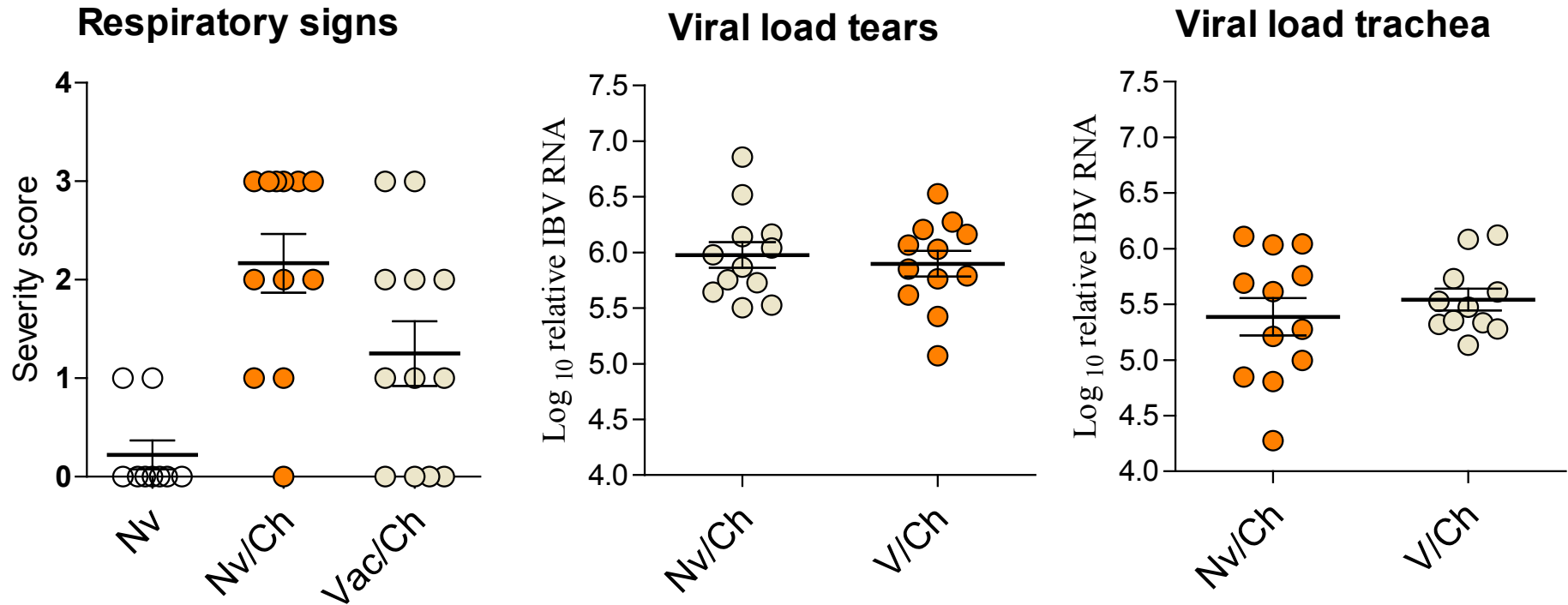
IBV RNA in tears



In 2018

In ovo Vaccination with Recombinant IBV S
Ectodomain Protein Delivered with Seppic Adjuvant
(Montanide IMS 1313 VG N ST)

Signs and viral load (IBV RNA) in tears and trachea of chickens vaccinated *in ovo* with S protein/adjuvant ^A (Montanide IMS 1313 VG N ST) and challenged ^B with virulent Ark IBV

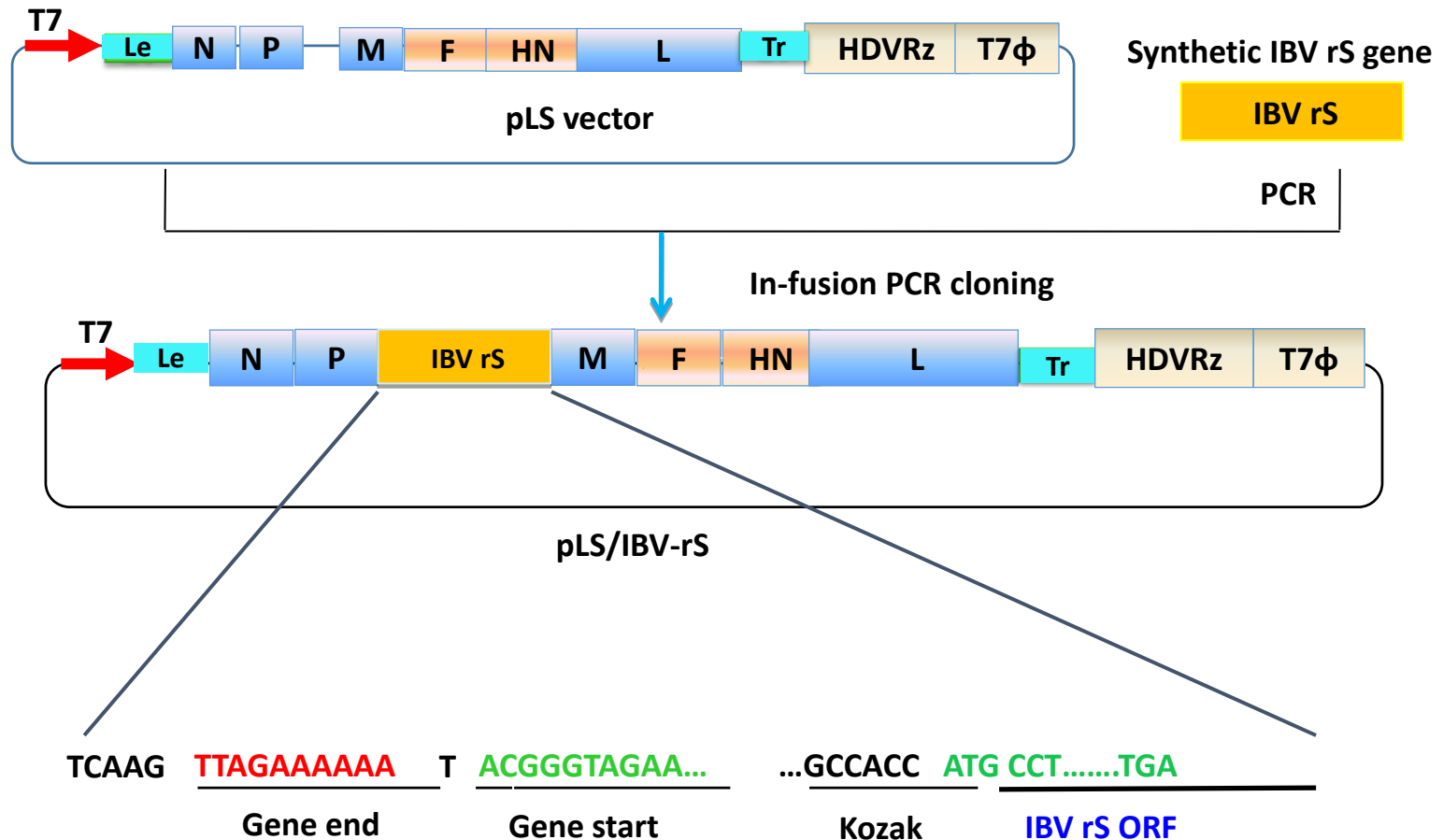


^A 50µg of protein diluted 1:1 in adjuvant / chicken egg.

^B 10⁵ EID₅₀ virulent Ark at 21 days of age

Generation of recombinant NDV with S ectodomain gene of IBV ArkDPI

Yu et al, World J. Vaccines 3:130-139, 2013



IBV ArkDPI population C2 S ectodomain gene (GenBank accession ABY66333) was synthesized with codon optimized for chicken.

In 2018

- **Confirmed the fidelity of rLS/IBV-S by sequencing.**
- **Titrated rLS/IBV-S by EID₅₀ and TCID₅₀.**
- **Detected IBV S protein expression by immunofluorescence.**
- **Determined the pathogenicity of rLS/IBV-S by MDT and ICPI.**
- **Evaluated protection conferred by rLS/IBV-S.**

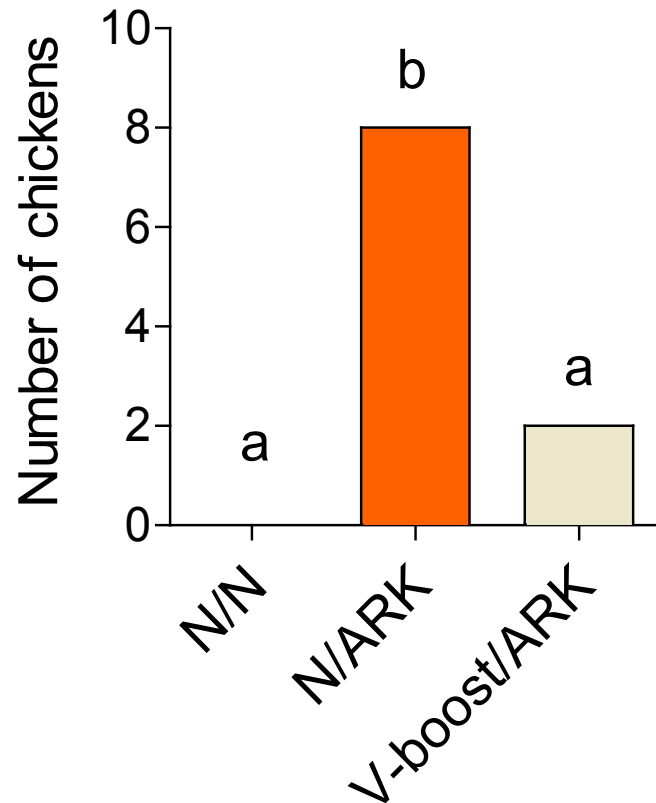
Properties of recombinant rLS expressing recombinant soluble trimeric IBV S-ectodomain protein.

rLS.S had an intracerebral pathogenicity index (ICPI) in day-old chickens of 0.0 and mean death time in embryonating eggs >150 hours, indicating even lower pathogenicity than the already low pathogenicity of the parental LaSota virus.

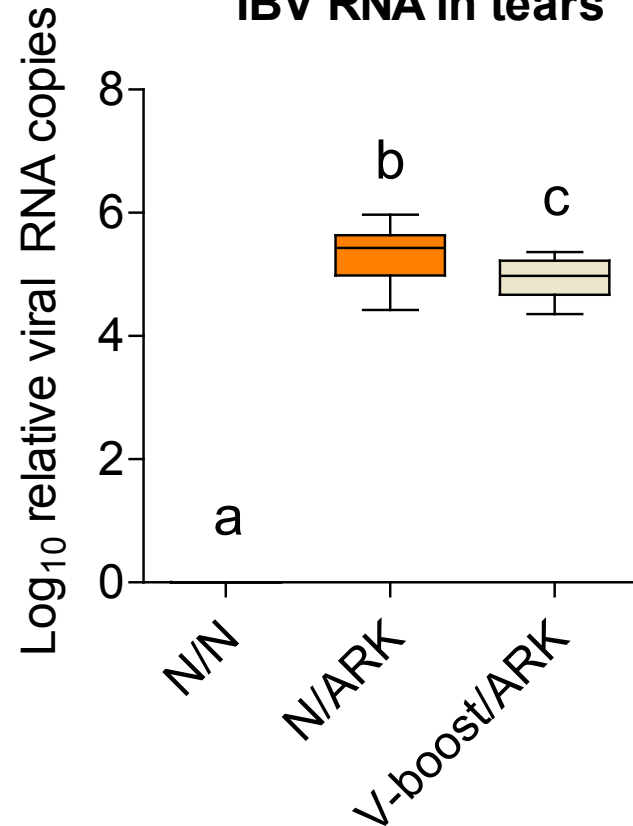
Expression of the recombinant S protein in NDV.S-infected cells *in vitro* was confirmed by indirect immunofluorescence using sera both from chickens vaccinated with Ark-type live vaccine and chickens vaccinated with recombinant Ark-type S-ectodomain protein

Incidence of signs and viral load in tears of chickens vaccinated ocularly at 1 and again at 14 days of age with rLS encoding S ectodomain and challenged^A with Ark

Incidence of respiratory signs

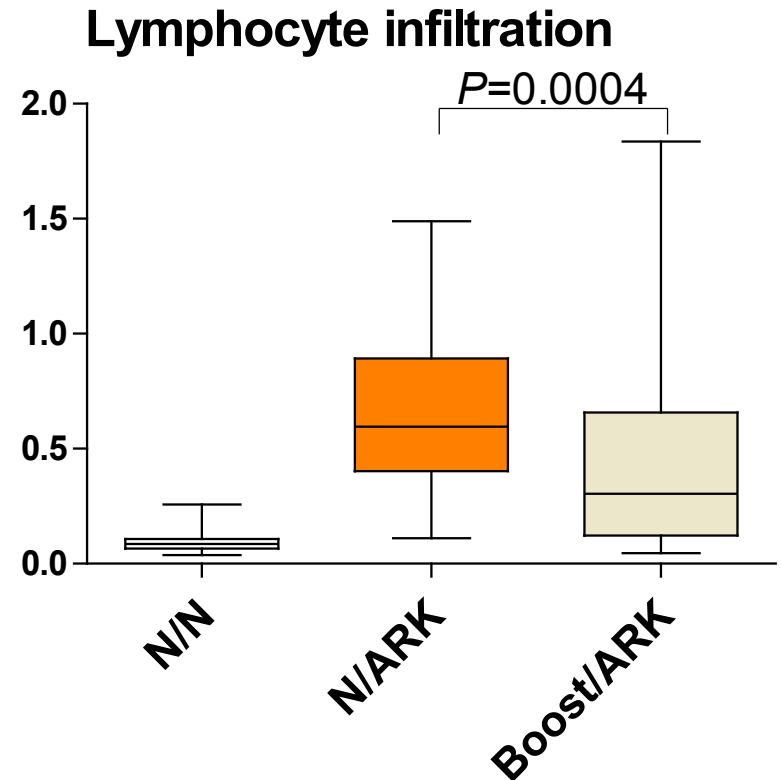
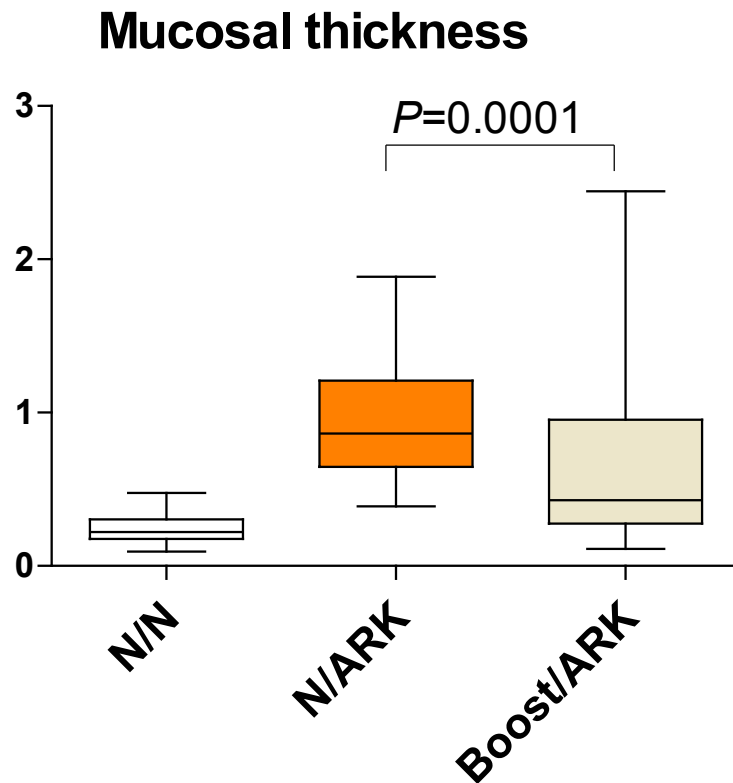


IBV RNA in tears



Vaccination: rLS.Se 10^5 EID₅₀ ; challenge: 10^6 EID₅₀ virulent Ark 18 days after 2nd vaccination

Tracheal histomorphometry after Ark challenge. Chickens vaccinated ocularly at 1 day-old and boosted on day 14 with rLS encoding IBV S ectodomain



Two tailed t test.

SCIENTIFIC REPORTS



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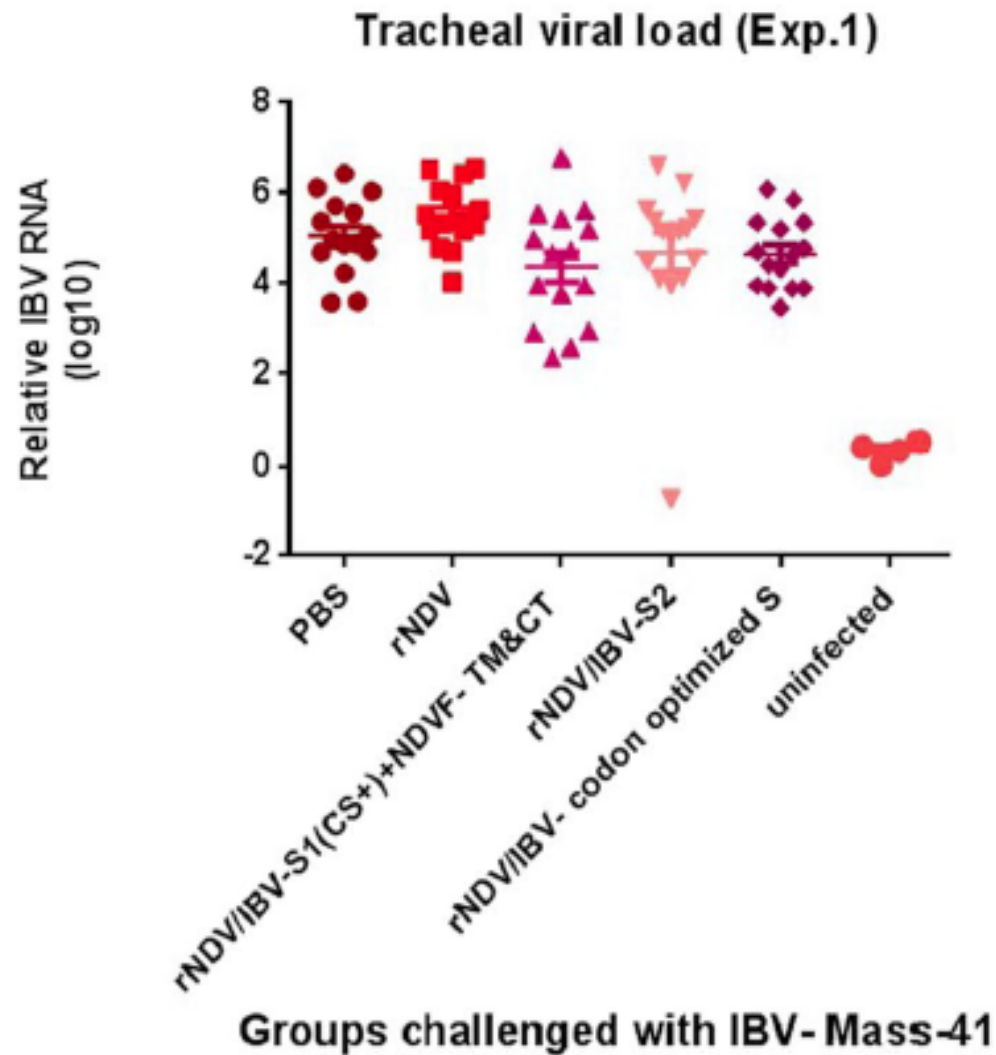
A Recombinant Newcastle Disease Virus (NDV) Expressing S Protein of Infectious Bronchitis Virus (IBV) Protects Chickens against IBV and NDV

Received: 4 January 2018

Accepted: 27 July 2018

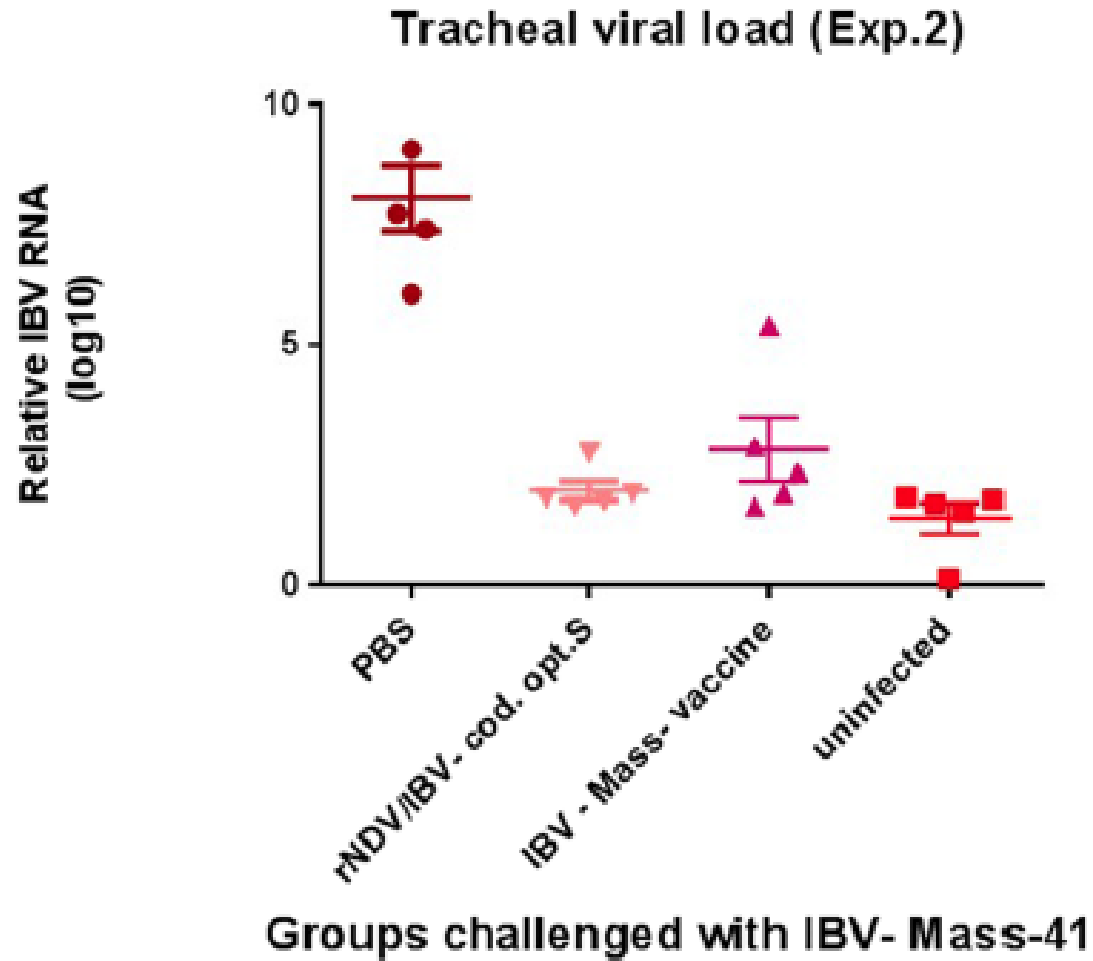
Published online: 10 August 2018

Edris Shirvani, Anandan Paldurai, Vinoth K. Manoharan, Berin P. Varghese & Siba K. Samal



Vaccinated at 1-d-old 10^7 EID₅₀

Challenge 3 weeks PV with $10^{3.1}$ EID₅₀ Mass



Vaccinated at 4 weeks old 10^7 EID₅₀

Challenge 3 weeks PV with $10^{3.1}$ EID₅₀ Mass

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