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College of Veterinary Medicine

S2 Expressed from Recombinant Virus to Confer Broad Protection against IBV

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S2 aa sequence identity among U.S. IBV types

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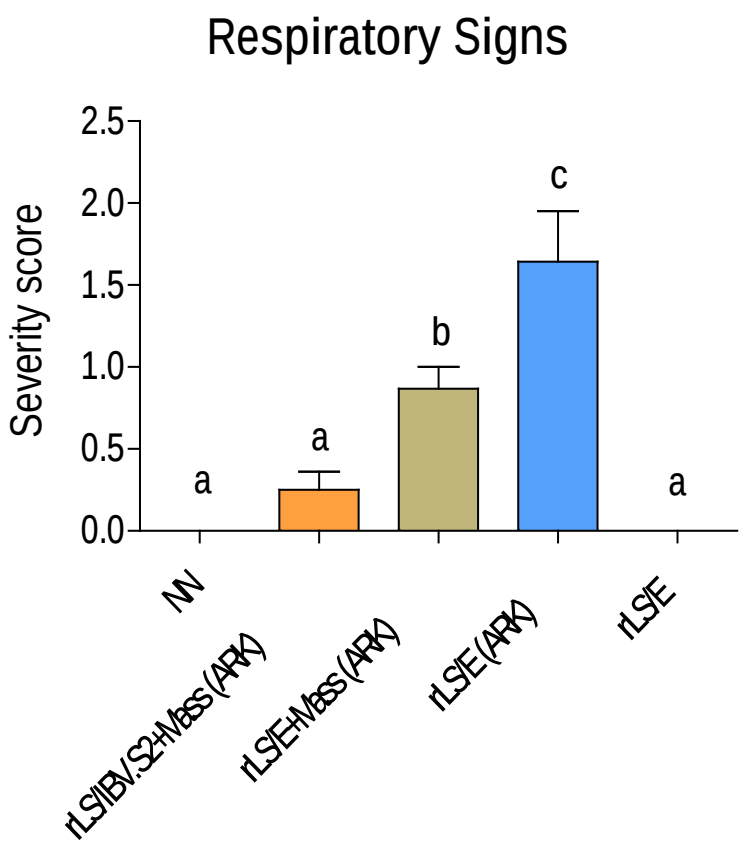
IDENTITY (%)

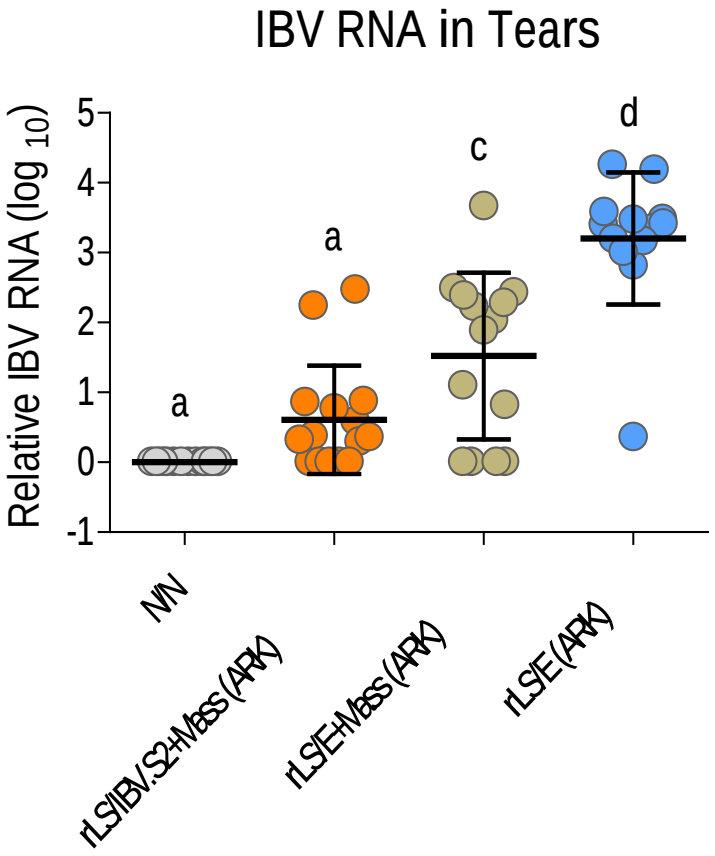
	Cal 95-9437	Cal 99	Cal 557 2003	CAV 56b	CAV 1013	Ark DPI	M41	DE 072	Conn 46	GA98	FL 18288	Md27	Iowa	Holte	Gray	JMK	GAV 92	Ark99
Cal 95-9437		98.4	94.4	96.8	94.9	91.4	89.8	75.7	91.2	76.2	90.9	90.9	91.4	91	91	91.4	92	92
Cal99	99		95.5	98.1	95.2	91.7	90.1	76.3	91.5	76.8	91.2	91	91.7	91.5	91.4	91.7	92.2	92.3
Cal557 2003	97.6	98.2		95.2	93.3	89.8	88.8	75.7	90.4	76.2	90.1	89.4	90.6	91	89.8	89.8	90.1	90.6
CAV 56b	98.2	98.9	97.8		94.2	90.9	89.1	76.6	90.7	77.1	90.4	90.1	90.7	90.6	90.4	90.9	91	91.4
CAV 1013	97.3	97.9	97.4	97.4		92.6	89.8	76.2	92.2	76.6	91.8	92	90.9	91.7	92.5	92.6	93.6	93.9
ArkDPI	95.5	96.2	95.7	95.7	96.8		94.6	75.7	95.8	75.5	95.5	97.8	94.1	94.9	98.9	99.7	93.6	93.9
M41	94.9	95.5	94.9	94.7	95.4	97.4		74.4	94.4	74.4	94.2	93.6	94.2	94.4	94.1	94.2	90.1	90.4
DE072	87	87.7	87.5	88	87.4	86.7	86.2		76.6	98.1	76.3	75.4	74.9	75.8	75.8	75.7	76.2	76.3
Conn 46	95.8	96.5	96.3	96	96.8	97.9	97.3	87.4		76.5	99.7	95.7	95.4	94.1	95.7	95.8	92.6	93
GA98	87.5	88.2	88	88.5	87.8	86.6	86.1	98.4	87.2		76.2	75.4	74.7	75.7	75.7	75.5	76.6	76.8
FL18288	95.7	96.3	96.2	95.8	96.6	97.8	97.1	87.2	99.8	87		95.4	95	93.8	95.4	95.5	92.3	92.6
Md27	95.5	96.2	95.8	95.5	97	98.7	96.8	86.9	97.6	86.9	97.4		93.4	94.1	97.4	97.8	93.6	94.1
Iowa	95.5	96.2	95.7	95.4	95.7	96.8	97	86.2	97.4	86.1	97.3	96.5		94.9	93.9	94.1	89.8	90.1
Holte	95.8	96.5	96	95.7	96.3	97.8	97.6	86.6	97.8	86.4	97.6	97.4	97.8		94.6	94.9	90.9	91.2
Gray	95.2	95.8	95.5	95.4	96.5	99.4	96.8	86.6	97.6	86.4	97.4	98.6	96.5	97.4		99.2	93.4	93.8
JMK	95.5	96.2	95.7	95.7	96.8	99.7	97.1	86.7	97.9	86.6	97.8	98.7	96.8	97.8	99.7		93.6	93.9
GAV92	95.2	95.8	95.8	95.4	96.8	97.1	95.7	86.2	96.5	86.7	96.3	97.1	95.4	95.8	96.8	97.1		98.9
Ark99	95.2	95.8	95.8	95.2	96.6	97	95.5	86.1	96.3	86.6	96.2	97.1	95.2	95.7	96.8	97	99.4	

SIMILARITY (%)

Toro et al., Avian Diseases 58:83-89, 2014

Priming with rLS virus encoding IBV S2 protein [≥ 98.7 % aa identity with IBV Ark] followed by boost with attenuated Mass-type vaccine confers protection against virulent Ark-type challenge.





Toro et al, Avian Diseases 58:83-89, 2014

rLS vectoring a custom-made consensus S2 transgene representing U.S. IBV types

- Consensus sequence (with threshold set at 51%) of 18 S2 aa sequences after aligning the sequences using ClustalW (Pairwise % identity ranged from 74.4-99.7%.)
- Pairwise percent identities ranged from 88.8%-99.7%, except for DE072 and GA98
- In 24 of 625 positions (3.8%), no single aa was found in a majority of sequences. In those positions, the aa found in the highest proportion of sequences was chosen for the consensus [aa found in 7-9 of the 18 sequences (39-50%)].
- At some positions only two aa were present, each at a frequency of 50%, and one of the two was chosen.
- The resulting S2 amino acid consensus sequence had ≥93% aa identity with the S2 sequences of most of the US serotypes (*except DE072 and GA98*).

S2 sequences of 18 U.S. IBV isolates	
TYPE	ACCESSION NUMBER
ArkDPI	ACB59372.1
Ark99	ABE68839.3
JMK	AAF64462.1
Gray	AAK77543.1
Conn 46	AAD34715.1
Holte	AAK20887.1
Fla 18288	AAD34716.1
Iowa	AAK20886.1
M41	AAW33786.1
Md27	ACM45229.1
Cal99	AAS00080.1
GAV 92	AAD34714.1
CAV 1013	AAF64465.1
CAL 95-9437	AAK28144.1
CAV 56b	AAK20888.1
Cal557	ADA83490.1
DE072	AAF08315.1
GA98	ADP06481.1

Objectives

(1) Further evaluate protection and immunity elicited by rLS encoding IBV S2 proteins.

(2) Optimize protection in chickens by rLS encoding distinct IBV S2 proteins.

We propose to develop and evaluate rLS vectoring the S2 gene of IBV UK4/91 (serotype 793/B)(13). Accumulating evidence indicates that the attenuated IBV 4/91 vaccine used in a combined prime-boost regime with attenuated Mass confers broad protection against IBV challenge (4,14). The lack of S1 sequence similarity between IBV 4/91 and the IBV strains against which it protects (e.g. the phylogenetically distant Chinese IBV QX type) (14) indicates that its cross-protection capabilities likely reside in immunodominant epitopes on the S2 protein. In brief, the S2 sequence of IBV 4/91 (GenBank accession #AEL97578.1) will be optimized to the chicken codons and synthesized. The rLS/IBV4/91.S2 will be developed as described (18).

Progress on generation of recombinant NDV containing IBV S2 gene of UK 4/91

- Infectious bronchitis virus (IBV) UK 4/91 strain S2 gene was synthesized with codon optimized for chicken.
- The IBV S2 gene has been cloned into the Newcastle disease virus (NDV) LaSota (LS) vaccine vector.
- The full-length cDNA clone of pLS/IBV-S2 are being sequenced to confirm its sequence fidelity.
- The infectious clone of pLS/IBV-S2 will be used for rescue of the rLS/IBV-S2 virus by using reverse genetics technology.

Progress on further evaluating protection and immunity elicited by rLS encoding IBV S2 proteins

rLS/S2+Mass & Mass+Mass vs. GA13

aa identity S1: M41 vs GA13= 75.6%

Experimental design				
Prime (3 DOA)	**rLS/S2**	**Mass**	**rLS/E**	**N/N**
Boost (16 DOA)	**Mass**	**Mass**	**-**	**-**
Challenge (30 DOA)	**GA13**	**GA13**	**GA13**	**-**

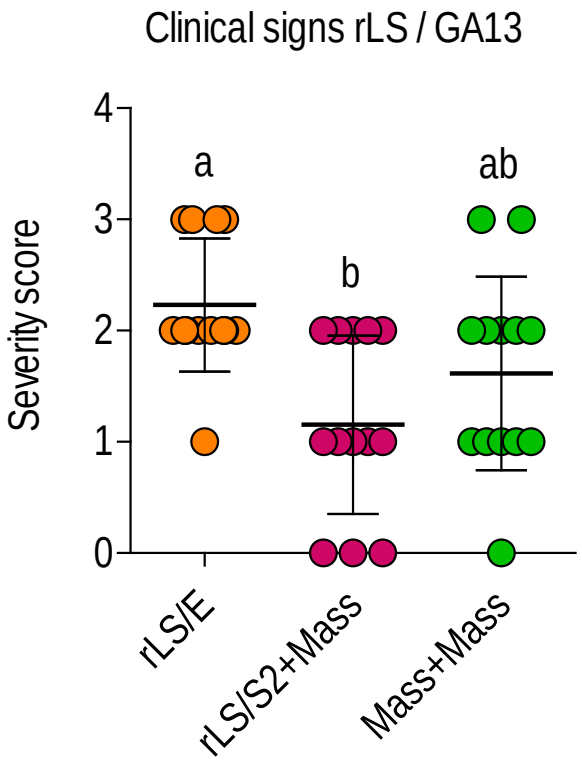
DOA=Days of age

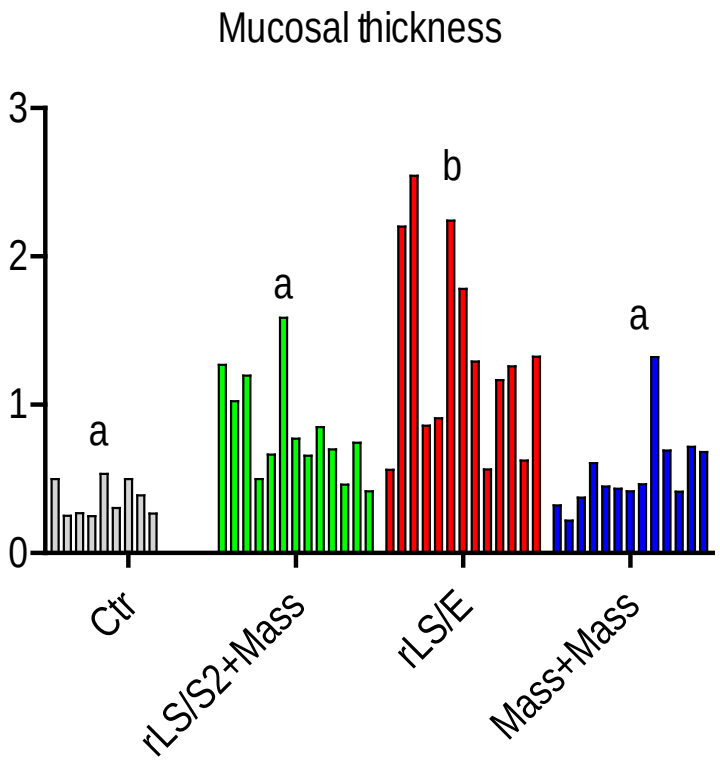
chickens/group = 13

Mass vaccine: 1 dose/bird (100ul).

rLS/S2+Mass & Mass+Mass vs. GA13

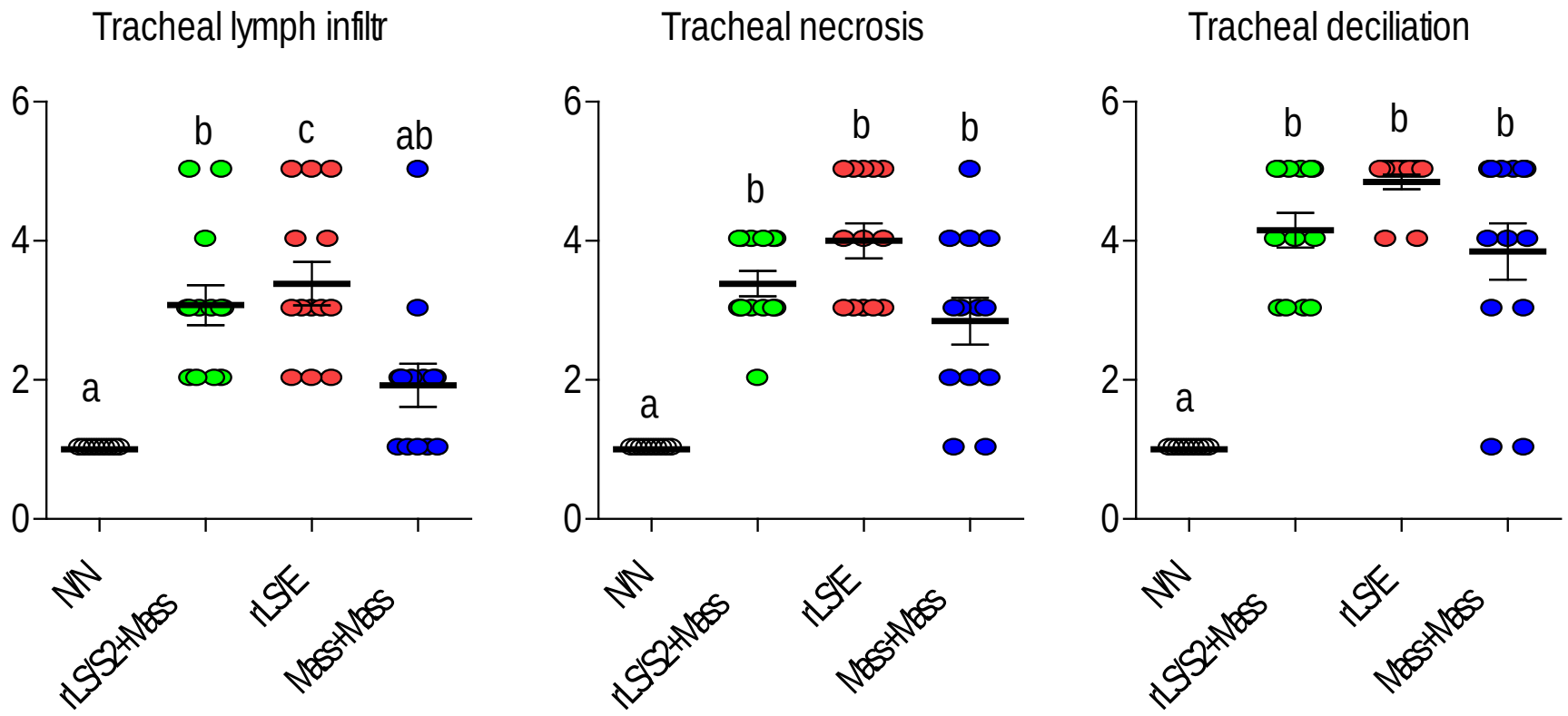
Clinical signs and histomorphometry





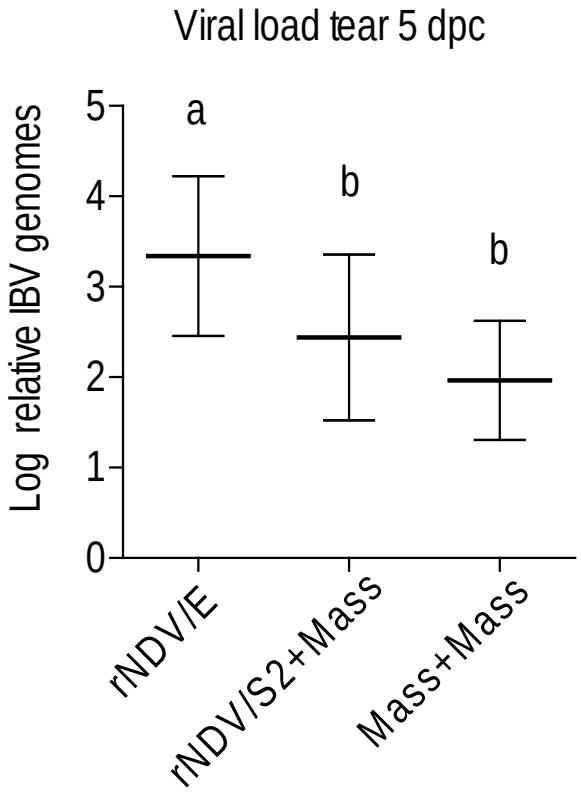
rLS/S2+Mass & Mass+Mass vs. GA13

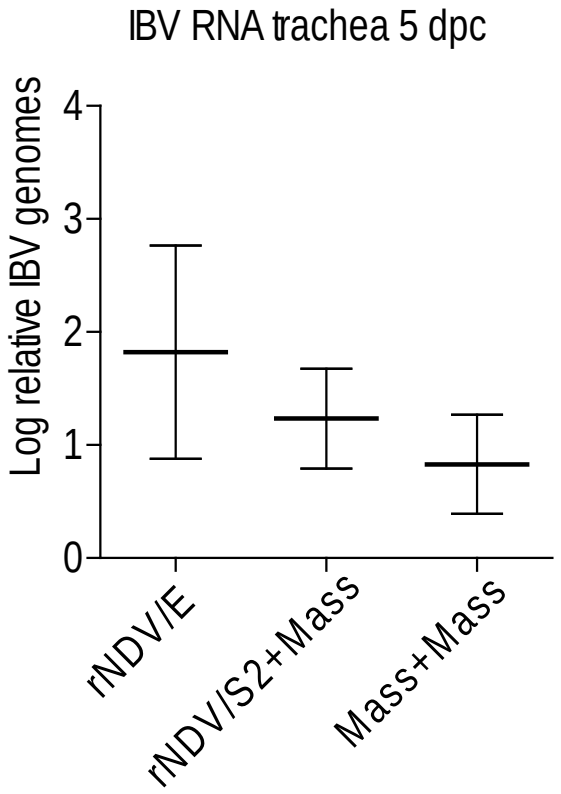
Tracheal histopathology



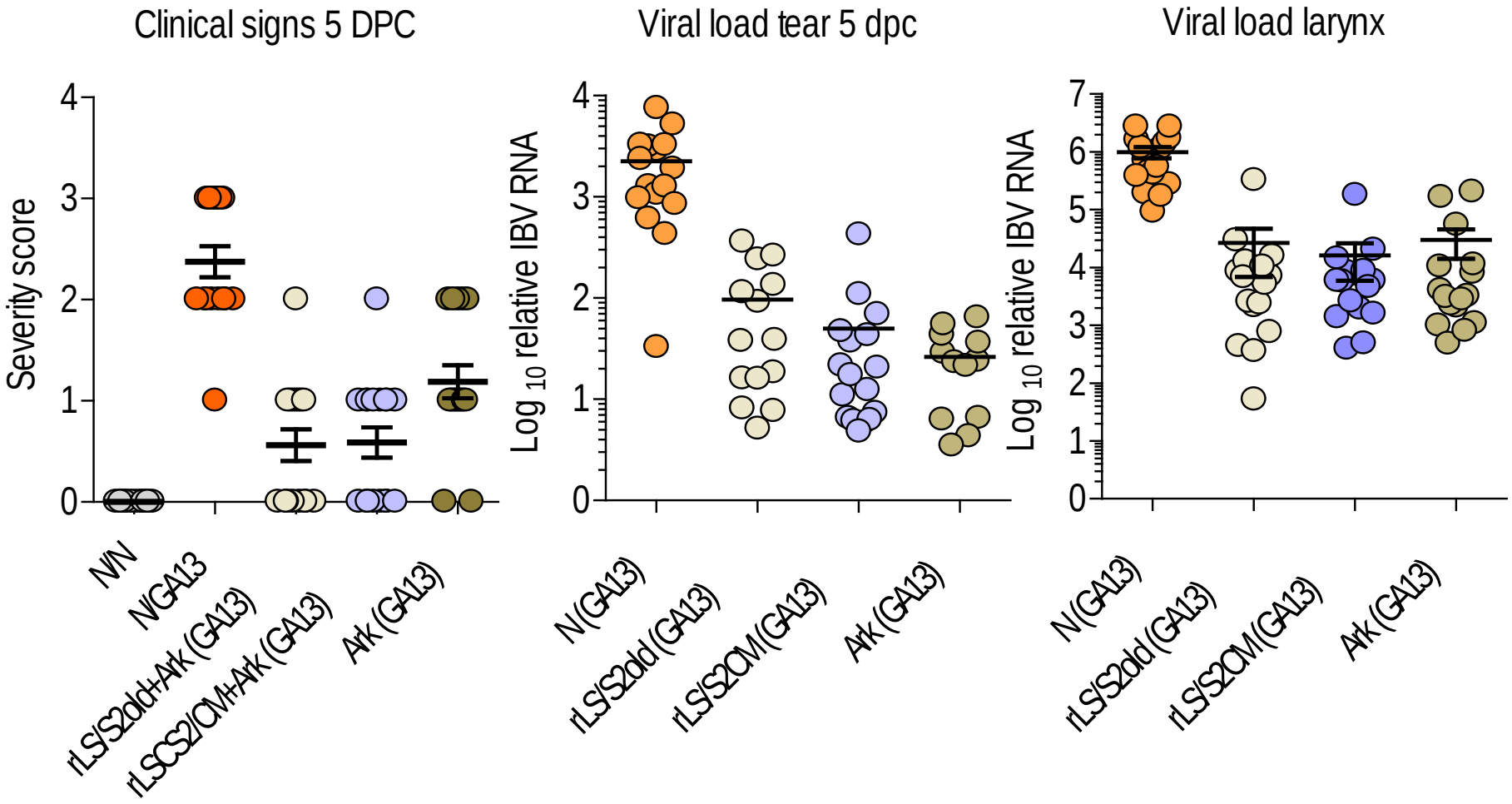
rLS/S2+Mass & Mass+Mass vs. GA13

Viral load





rLS/S2Ark, rLS/S2cons, or Ark vs. GA13



n=16 chickens/group

Two IB vaccine concept

- When a live IB vaccine from one serotype is followed by vaccination with an IB variant from another serotype, birds develop immunity to the serotypes in both vaccines – in addition to cross-reacting antibodies to other IB serotypes.
- By using the right combination of IB vaccines, existing vaccines protect poultry against several of the IB-variant viruses.

Cook JKA, Orbell SJ, Woods MA, Huggins MB (1999). Avian Pathology 28:477-485.

Terregino C, Toffan A, Beato MS, De Nardi R. Vascellari M, Meini A (2008). Avian Pathology, 37, 487-493.

Is there strong scientific support for this concept?

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Terregino et al (2008). Avian Pathology, 37:487-493.

Type of bird	Vaccination 1 day	Vaccination 14 days	35 days QX-challenge
SPF	Ma5	4/91	+
Broiler	Ma5	4/91	+
SPF	n/v	n/v	+
Broiler	n/v	n/v	+
3 SPF; 3 broiler	n/v	n/v	-

Missing CONTROLS!!!!!

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Unfortunately the *two vaccine* concept has been used to introduce exotic IBV vaccine strains into free regions

A protocol using an Mass vaccine on day 1 followed by booster vaccination with IB 4/91, a variant originating in the UK (now common throughout Europe), has been introduced in several countries; most recently in Latin America.

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Cross-Protection by Infectious Bronchitis Viruses Under Controlled Experimental Conditions

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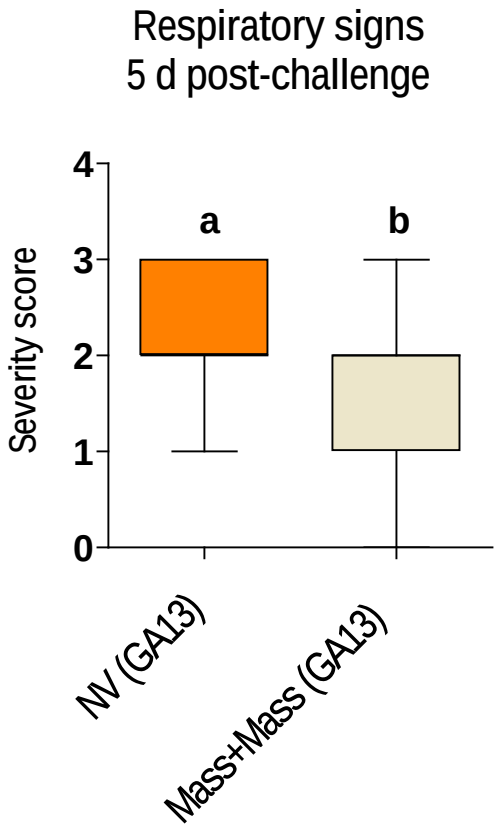
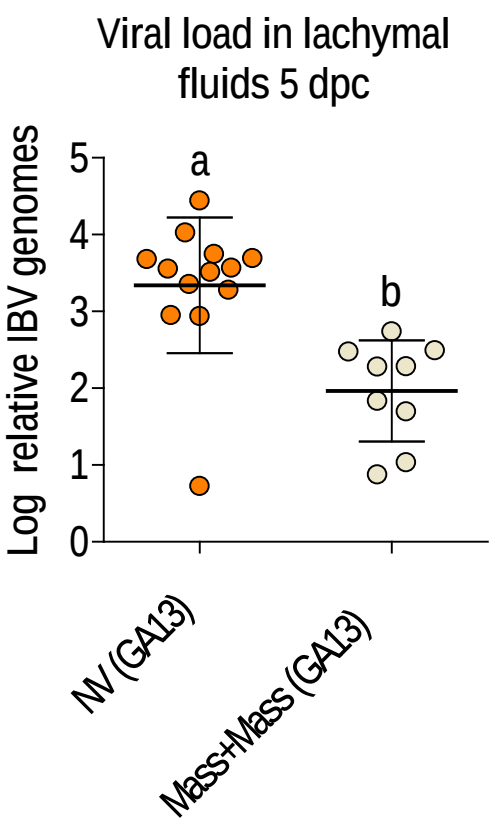
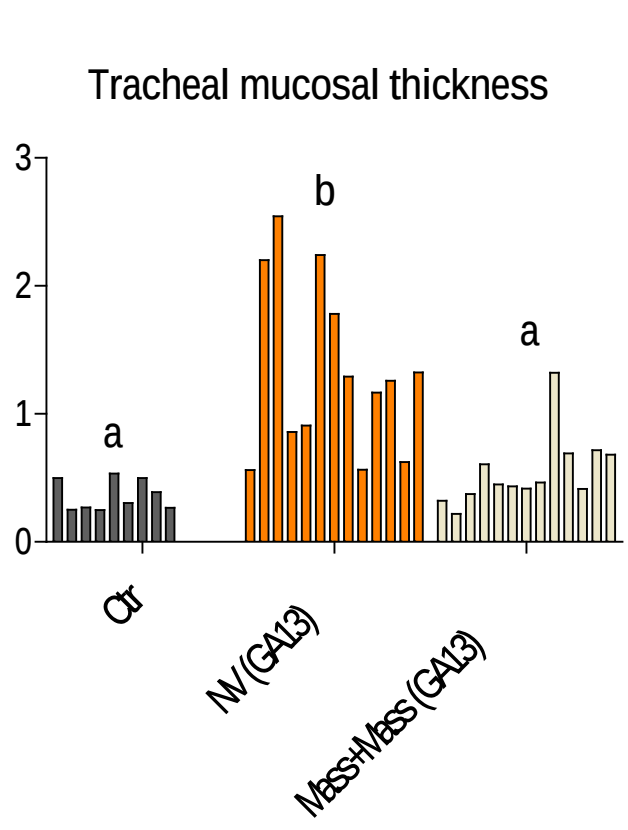
SUMMARY. Infectious bronchitis virus (IBV) cross-protection trials were performed in healthy chickens maintained under controlled environmental conditions. Chickens primed or primed and boosted with a Massachusetts (Mass)-type attenuated vaccine were subsequently challenged with either IBV Arkansas (Ark) or GA13-type virulent strains. In addition, Ark-vaccinated chickens were challenged with IBV GA13. Spike protein 1 (S1) amino acid identities between IBV vaccine and challenge strains varied from 76.0% to 77.3%. Contrary to expectations, assessments of clinical signs, viral load, and histopathology indicated a significant level of cross-protection among these antigenically distant IBV strains. Moreover, prime and booster vaccination with Mass protected against GA13 and improved protection against Ark when compared with Mass single vaccination. These results emphasize the need to include both single vaccination control groups and control groups primed and boosted with a single serotype when testing the efficacy of IBV protectotypes and/or novel IBV vaccine combinations against heterologous serotypes under controlled experimental conditions. Such controls are of distinct importance in experiments supporting the introduction of attenuated IBV vaccine strains exotic to regions, since these exotic strains may provide new genetic material for recombination and emergence of novel IBV strains.

Protection by priming and boosting with a Mass-type attenuated vaccine against IBV GA13 *under experimental conditions*







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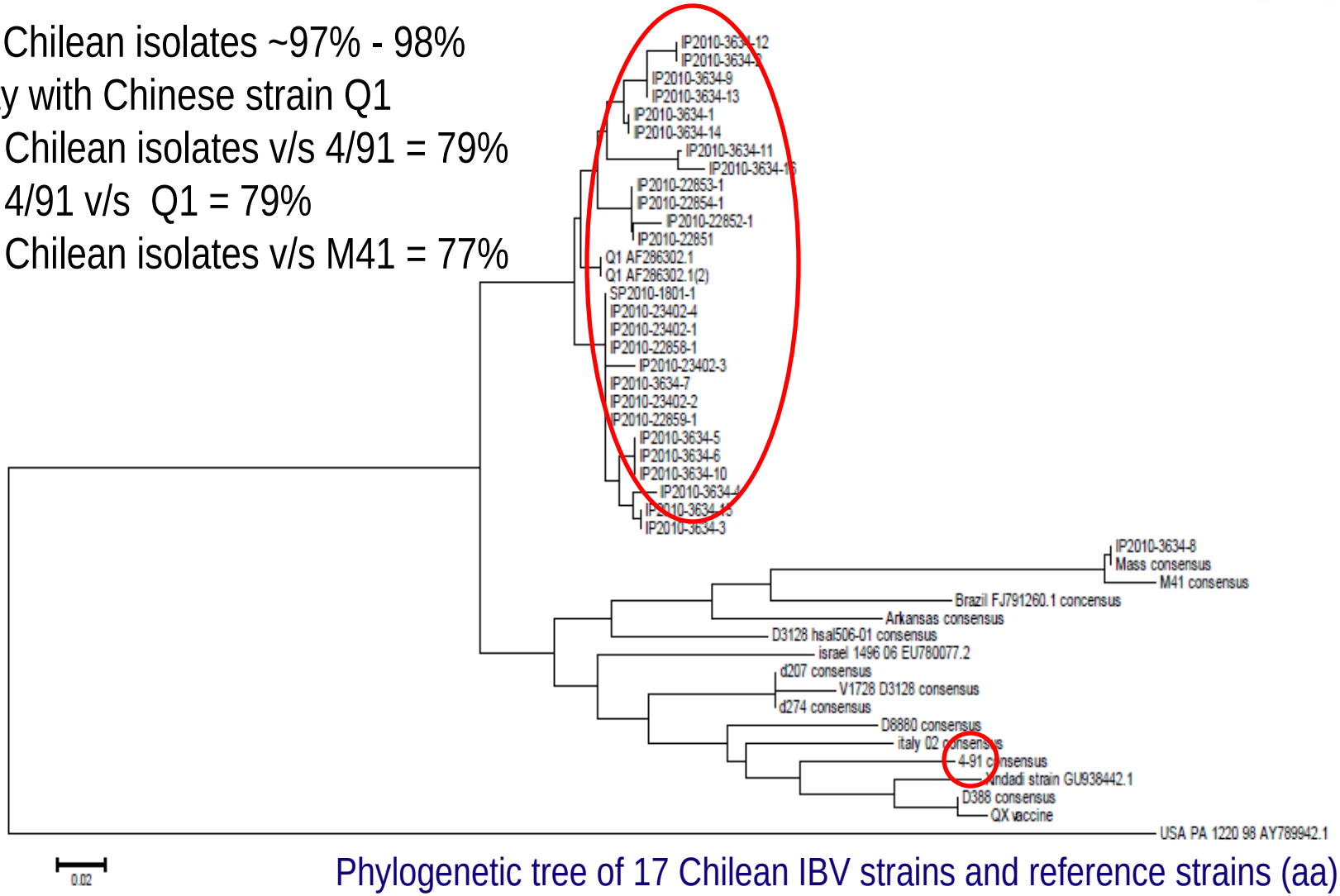
Introduction of exotic IBV 4/91 in Chile

- Cluster Chilean isolates ~97% - 98% similarity with Chinese strain Q1
- Identity Chilean isolates v/s 4/91 = 79%
- Identity 4/91 v/s Q1 = 79%
- Identity Chilean isolates v/s M41 = 77%



Phylogenetic tree of 17 Chilean IBV strains and reference strains (aa)

(figure from J. J. DE WIT et al, 2012)

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Vaccination/challenge experiment with 5 Chilean strains (de Witt et al 2012)

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Strain	Vaccination		Challenge at day 33, 34 or 35	Ciliostasis protection score 5 dpc	Percent of 10 birds with detectable IBV antigen in the kidney by IHC			
	Day of hatch	Day 14			Kidney		Proventriculus	
Experiment A					**5 dpc**	**8 dpc**	**5 dpc**	**8 dpc**
Negative control	-	-	No	100	0	0	0	0
IP2010-03634-2	-	-	Yes	0	50	40	0	0
	MA5	-	Yes	44	30	40	0	0
	MA5	4/91	Yes	100	0	10	0	0
IP2010-03634-6	-	-	Yes	4	20	0	0	0
	MA5	-	Yes	76.5	10	0	0	0
	MA5	4/91	Yes	100	0	0	0	0
IP2010-03634-7	-	-	Yes	5.5	10	20	0	0
	MA5	-	Yes	92.5	0	0	0	0
	MA5	4/91	Yes	99	0	0	0	0
IP2010-03634-12	-	-	Yes	33	0	0	0	0
	MA5	-	Yes	97.5	Nt	Nt	Nt	Nt
	MA5	4/91	Yes	99	Nt	Nt	Nt	Nt
Experiment B								
Negative control		No	No	98	0	0	Nt	Nt
D10-273-4	-	-	Yes	0	20	20	Nt	Nt
	MA5	-	Yes	58.5	0	0	Nt	Nt
	MA5	4/91	Yes	100	0	0	Nt	Nt

(figure from DE WIT et al, 2012)

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Missing CONTROLS

Efficacy of MA5 and 4/91 vaccines applied in combination or separately against a D388 (QX genotype), Q1, and variant 2 challenge (de Wit et al, 2014).

Group	Vaccination		Challenge strain	Ciliostasis protection score	Percentage of IBV IHC negative kidneys 5 and 8 d.p.c.*	
	Day 1	Day 14	Day 28	5 d.p.c.	5 d.p.c.	8 d.p.c.
1	No	No	No	99	100	100
2			D388	0	40	20
3			Q1	0	40	0
4			Var2	26	100	100
5	Ma5	4/91	D388	84	100	80
6			Q1	48	100	80
7			Var2	98	100	100
8	Ma5 + 4/91	No	D388	70	70	50
9			Q1	96	90	90
10			Var2	97	70	100

10 birds were examined at 5 d.p.c. (days post challenge)

Table from: J. J. de Wit, R. Koopman, L.Y. Villarreal (2014). 8th Symposium Acov & Ampv, Rauischholzhausen, Germany.

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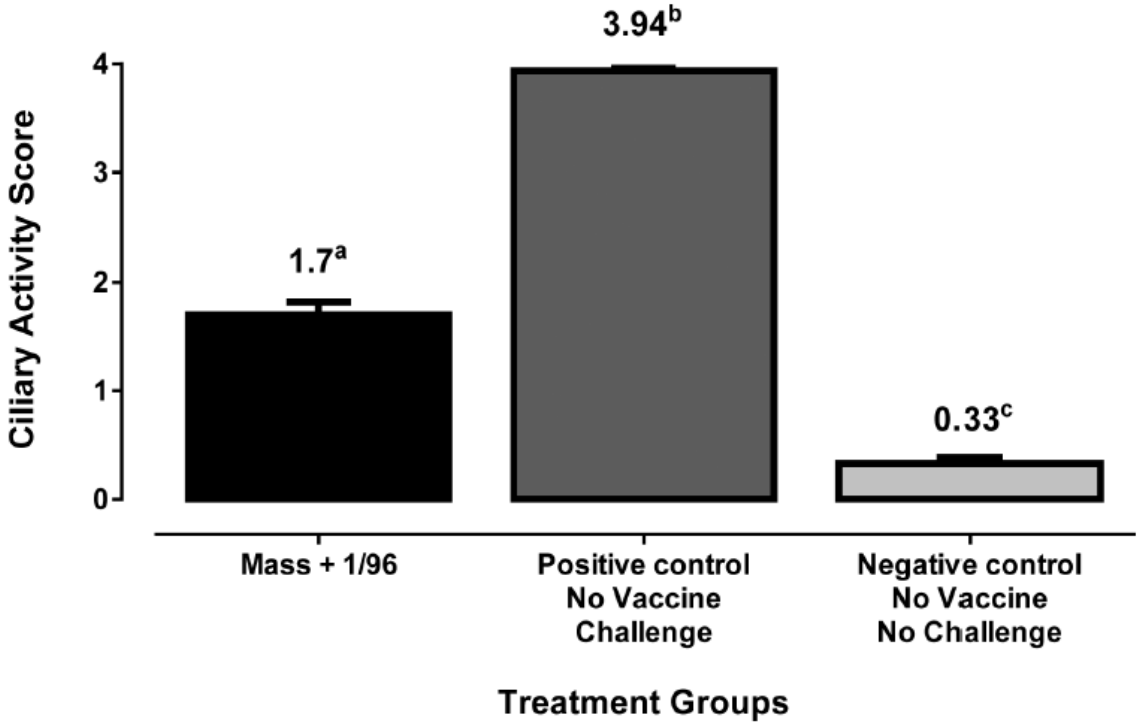
Introduction of 4/91 in Argentina

A vaccine combination trial for the control of the variant Q1 IBV in South America (Sesti et al 2014).

Missing CONTROLS!!



Ciliary activity test scores (low score =high ciliary activity): 0-2=protected; 3-4= not protected. Different letters=significant differences at $p<0.05$.

Extracted from Sesti et al (2014) 8th Symposium Acov & Ampv, Rauischholzhausen, Germany.

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Conclusions

- Evaluation of protection conferred by rLS is difficult because assessment of clinical signs, viral load, and histopathology indicate a significant level of cross-protection among antigenically dissimilar IBV when tested under environmentally controlled conditions in healthy chickens.
- Include both single vaccination control groups and primed and boosted with a single serotype the efficacy of IBV protectotypes under controlled experimental conditions.
- The concept that using the right combination of IBV vaccines confers heterotypic protection requires stronger scientific support.

This project was supported by Agriculture and Food Research Initiative Competitive Grant no. (2015-68004-23131) from the USDA National Institute of Food and Agriculture."