PROGRESS REPORT

AUBURN UNIVERSITY

College of Veterinary Medicine, Auburn, AL 36849

October 2015

PROJECT: Regional NC-1180, Control of Emerging and Re-emerging Poultry Respiratory Diseases in the United States

COOPERATIVE AGENCIES AND PRINCIPAL INVESTIGATORS

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OBJ. 1. IDENTIFY RESERVOIRS OF INFECTIOUS RESPIRATORY DISEASE AGENTS IN WILD BIRDS AND POULTRY.

Identify reservoirs of avian influenza virus (AIV) in beetles, drinking water, and mice.

Darkling beetle populations were harvested from used poultry litter and propagated under control conditions. The LP H1N1 of AIV was propagated and titrated in fertile SPF chicken embryos. The virus was sprayed on chicken feed and the beetles were allowed to feed on it. Attempts will be made to reisolate the virus from the beetles using embryos followed by the HI test and RT/RT-PCR.

J.J. Giambrone, K.S. Macklin (Auburn University).

OBJ. 3. INVESTIGATE THE PATHOGENESIS AND POLYMICROBIAL INTERACTIONS OF SPECIFIC INFECTIOUS AGENTS ASSOCIATED WITH POULTRY RESPIRATORY DISEASES (THIS INCLUDES INTERACTIONS WITH UNDERLYING IMMUNOSUPPRESSIVE AGENTS)

Variability Assessment of California Infectious Bronchitis Virus Variants.

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M41

	S1 sequence (nt, AA)						
Predominant population	**26 9**	**170 57**	**377 126**	**566 189**	**626 209**	**653 218**	**680 227**
M41 Field strain	Val	Asn	Leu	Leu	Tyr	Val	Gly
E	**Ala**	Asn	Leu	Leu	Tyr	Val	Gly
G	**Ala**	Asn	**Arg**	Leu	Tyr	Val	Gly
I	**Ala**	Asn	**Arg**	Leu	Tyr	Val	**Val**
J	**Ala**	Asn	**Arg**	**Phe**	Tyr	Val	Gly
K	**Ala**	Asn	**Arg**	Leu	**Cys**	**Asp**	Gly
L	**Ala**	**Thr**	**Arg**	Leu	Tyr	Val	Gly

Cal99

	S1 sequence (nt, AA)						
Predominant population	**12 4**	**74 25**	**284 95**	**400 134**	**635 212**	**637 213**	**638 213**
Cal 99 Field strain	Leu	Ser	Lys	Arg	Gln	Leu	Leu
M	**Phe**	**Phe**	**Thr**	Arg	**Arg**	Leu	**Pro**
N	Leu	**Phe**	Lys	Arg	Gln	Leu	Leu
P	Leu	**Phe**	**Thr**	Arg	**Arg**	Leu	Leu
Q	Leu	**Phe**	Lys	Arg	Gln	**Val**	Leu
S	Leu	**Phe**	**Thr**	Arg	Gln	**Val**	Leu
T	Leu	**Phe**	Lys	**Gly**	Gln	**Val**	Leu

Ark

	S1 sequence (nt, AA)											
Predominant population	**28 9**	**53 18**	**105 35**	**400 134**	**404 135**	**406 136**	**407 136**	**410 137**	**634 212**	**636 212**	**671 224**	**696 232**
Ark Field strain	Thr	Ala	Arg	Arg	Ile	Ala	Ala	Ala	Gln	Ser	Asp	Pro
1	Thr	Ala	Arg	Arg	Ile	Ala	Ala	Ala	**Lys**	Ser	Asp	Pro
3	Thr	Ala	Arg	Arg	Ile	Ala	Ala	Ala	**Lys**	**Thr**	Asp	Pro
4	Thr	Ala	Arg	Arg	Ile	Ala	Ala	Ala	**Lys**	Ser	Asp	**Ser**
8	Thr	**Val**	Arg	Arg	Ile	Ala	Ala	Ala	**Lys**	**Thr**	Asp	Pro
9	Thr	Ala	Arg	Arg	Ile	Ala	**Val**	Ala	**Lys**	**Thr**	Asp	Pro
10	Thr	Ala	Arg	Arg	Ile	**Pro**	Ala	**Val**	**Lys**	**Thr**	Asp	Pro
11	Thr	Ala	Arg	Arg	Ile	Ala	Ala	**Val**	**Lys**	**Thr**	Asp	Pro
12	**Pro**	Ala	Arg	Arg	Ile	Ala	Ala	Ala	**Gln**	**Ser**	Asp	Pro
13	Thr	Ala	Arg	Arg	Ile	Ala	Ala	Ala	**Lys**	**Thr**	**Ala**	Pro
14	Thr	Ala	Arg	Arg	**Asp**	Ala	Ala	Ala	**Lys**	**Thr**	**Ala**	Pro
16	Thr	Ala	Arg	**Cys**	Ile	Ala	Ala	Ala	**Lys**	**Thr**	**Ala**	Pro
18	Thr	Ala	**Ser**	Arg	Ile	Ala	Ala	Ala	**Lys**	**Thr**	**Ala**	Pro

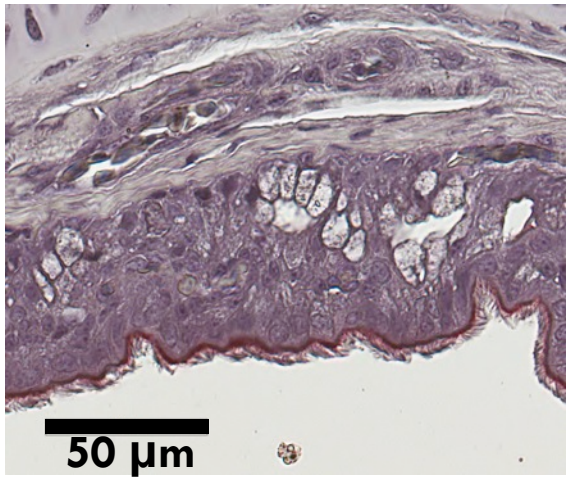
CAV1737

	S1 sequence (nt, AA)						
Predominant population	**167 56**	**271 91**	**389 130**	**407 136**	**619 207**	**620 207**	**647 216**
CAV Field strain	Ser	Ser	Gln	Ser	Gln	Gln	Tyr
1	Ser	**Arg**	Gln	Ser	Gln	**Arg**	Tyr
2	Ser	**Arg**	Gln	Ser	Gln	**Pro**	Tyr
3	Ser	**Arg**	Gln	Ser	Gln	**Pro**	**Asn**
5	Ser	**Arg**	Gln	**Phe**	Gln	Gln	Tyr
6	Ser	**Arg**	Gln	Ser	**Lys**	Gln	**Asn**
7	Ser	**Arg**	Gln	Ser	Gln	Gln	Tyr
8	Ser	**Arg**	Gln	Ser	Gln	Gln	**Asn**
10	**Tyr**	Ser	Gln	Ser	Gln	Gln	Tyr
15	Ser	**Arg**	**Pro**	Ser	Gln	Gln	Tyr

R A Gallardo, OA Aleuy, M Pitesky, G Senties-Cue, A Abdelnabi, P R Woolcock, MR Hauck, H Toro (UC, Davis & AU).

Role of differences in spike protein in selection of IBV ArkDPI vaccine subpopulations

Trachea



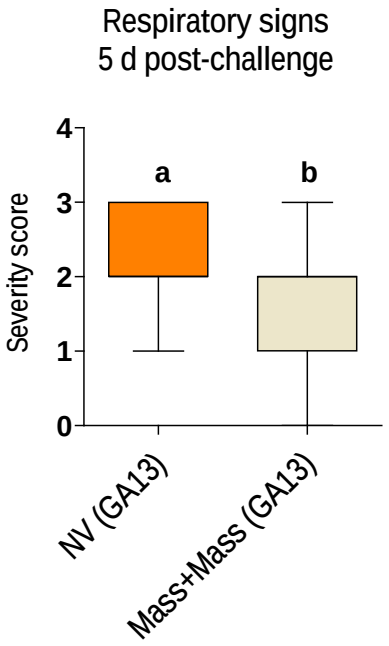
S1 of vaccine major population

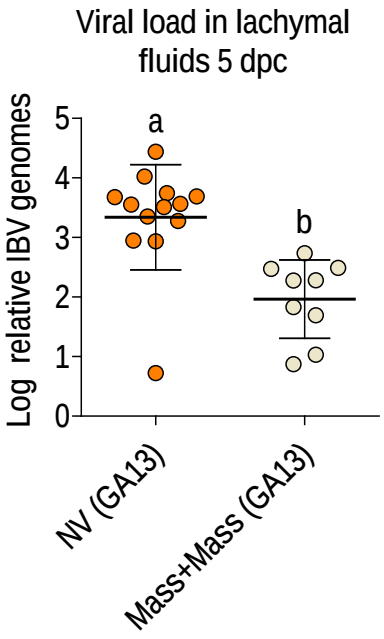
S1 of subpopulation selected in chickens

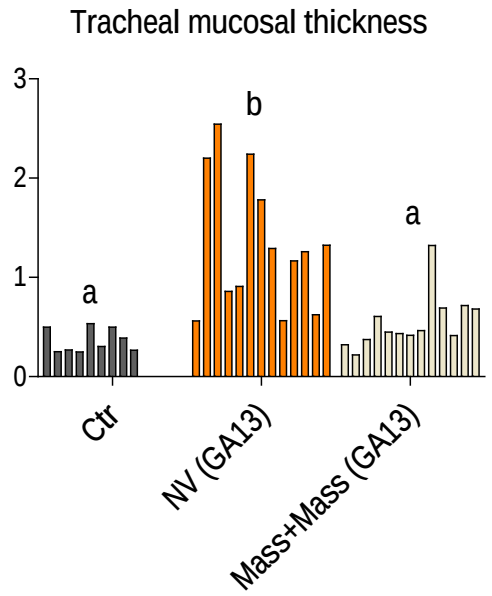
- Trimeric strep-tagged recombinant S1 proteins produced in eukaryotic cells (HEK293T)
  - Heavily glycosylated
- Binding to chicken tissues detected by Streptactin-HRPO and AEC
- S1 of this vaccine subpopulation selected in chickens binds better to tracheal epithelium and other relevant chicken tissues than S1 of major vaccine population
  - Not true for all selected subpopulations
- More efficient attachment might play a role in selection of some, but not all, ArkDPI vaccine subpopulations in chickens

V. L. van Santen, F. Eldemery, S. Farjana, K.S. Joiner, H. Toro
Auburn University

Cross-protection by Infectious Bronchitis Viruses under Controlled Experimental Conditions







Toro, H., V. L. van Santen, A. M. Ghetas, and K. S. Joiner (Auburn University).

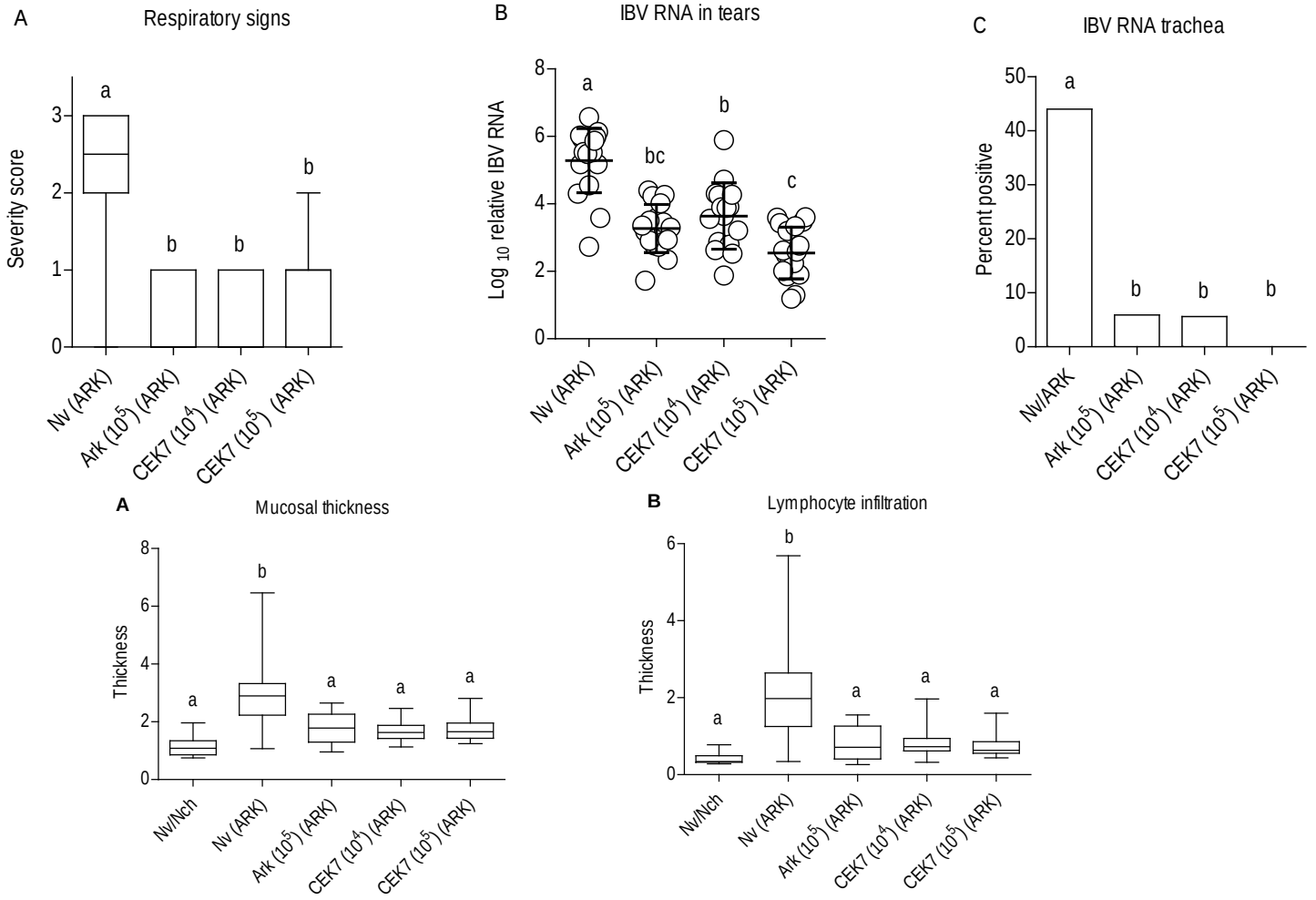
OBJECTIVE 4. DEVELOP NEW PREVENTION AND CONTROL STRATEGIES FOR POULTRY RESPIRATORY DISEASES

Effects of Adaptation of IBV Arkansas Attenuated Vaccine to Embryonic Kidney Cells

- CEK adaptation of embryo-attenuated Ark vaccines reduces population heterogeneity and changes do not revert after one replication cycle in ECE or in chickens. CEK adaptation provides an opportunity to improve commercial ArkDPI-derived vaccines.

Ghetas A.M., G.E. Thaxton, C. Breedlove, V.L. van Santen, H. Toro (Auburn University)

Kidney Cell-Adapted Infectious Bronchitis ArkDPI Vaccine Confers Effective Protection against Challenge



Ghetas A.M., V.L. van Santen, K. Joiner, and H. Toro (Auburn University).

Combined infectious bronchitis virus Ark and Mass serotype vaccination suppresses replication of Ark vaccine virus

- Chickens ocularly vaccinated with combinations of Ark and Mass showed predominance of Mass vaccine virus before 9 days post-vaccination (DPV) in tears.
- When chickens vaccinated with Ark or Mass vaccine were housed together, Mass vaccine virus was able to spread to Ark-vaccinated chickens, but the Ark vaccine was not detected in Mass-vaccinated chickens.
- Ark vaccine virus RNA was not detectable until 10 DPV in most tear samples from chickens vaccinated with both Ark and Mass vaccines at varying Ark vaccine doses, while high concentrations of Mass virus RNA were detectable at 3-7 DPV
- The different replication dynamics of Ark and Mass viruses in chickens vaccinated with combined vaccines did not result in reduced protection against Ark challenge at 21 days post vaccination.

E.N. Ndegwa, S. N. Bartlett, H. Toro, Kellye S. Joiner, V. L. van Santen, Auburn University.

IBV S2 Expressed from Recombinant Virus to Confer Protection across Serotypes in Chickens.

- rLS vectoring the S2 gene of IBV UK4/91 (serotype 793/B) was developed. The S2 sequence of IBV 4/91 (GenBank accession #AEL97578.1) was optimized to the chicken codons and synthesized.
- Several trials to investigate protection conferred by rLS/S2 were performed using GA13 as the challenge strain.

H. Toro, Q. Yu, V.L. van Santen, F.W. van Ginkel, K. Joiner (Auburn University)

Development of a recombinant vaccine against infectious laryngotracheitis virus (ILTV)

- Construction of recombinant NDV expressing ILTV-gB gene (rNDV-ILTV/gB) by plasmid-based reverse genetics techniques.

Giambrone. J., K. S. Macklin, and H. Wu (Auburn University & Alabama State University)

Development of a peptide vaccine against infectious bursal disease virus (IBDV)

- T cell vaccine platform that is based on immunization with low femtoMole doses of antigen peptides rather than whole protein antigens.
- The delivery platform for this vaccine is a powder of microspheres of 2 µm diameter composed of peptide antigens combined with a matrix of biodegradable poly (lactide-co-glycolide)-poly-ethylene-glycole copolymer (PLGA-PEG) and the block copolymer adjuvant Pluronic L121®.
- In two high-dose challenge infections the initial vaccine formulations were not protective. However, in a low-dose challenge infection advanced vaccine formulations provided 50 and 80% protection.

J. Giambrone, B. Kaltenboeck (Auburn University).

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