

# Effect of ambient ammonia on the immune response to infectious bronchitis virus vaccination and protection from homologous challenge in broiler chickens

<sup>1</sup>Emily J. Aston, <sup>1</sup>Mark W. Jackwood, <sup>2</sup>Robert M. Gogal, Jr., <sup>1</sup>Maricarmen Garcia, <sup>3</sup>Brian D. Fairchild, <sup>4</sup>David J. Hurley, <sup>1</sup>Deborah A. Hilt, <sup>1</sup>Sunny Cheng, <sup>1,3</sup>Brian J. Jordan

<sup>1</sup>Poultry Diagnostic and Research Center, Department of Population Health, College of Veterinary Medicine, University of Georgia, <sup>2</sup>Veterinary Biosciences and Diagnostic Imaging, College of Veterinary Medicine, University of Georgia, <sup>3</sup>Department of Poultry Science, College of Agricultural and Environmental Sciences, University of Georgia, <sup>4</sup>Department of Population Health, College of Veterinary Medicine, University of Georgia

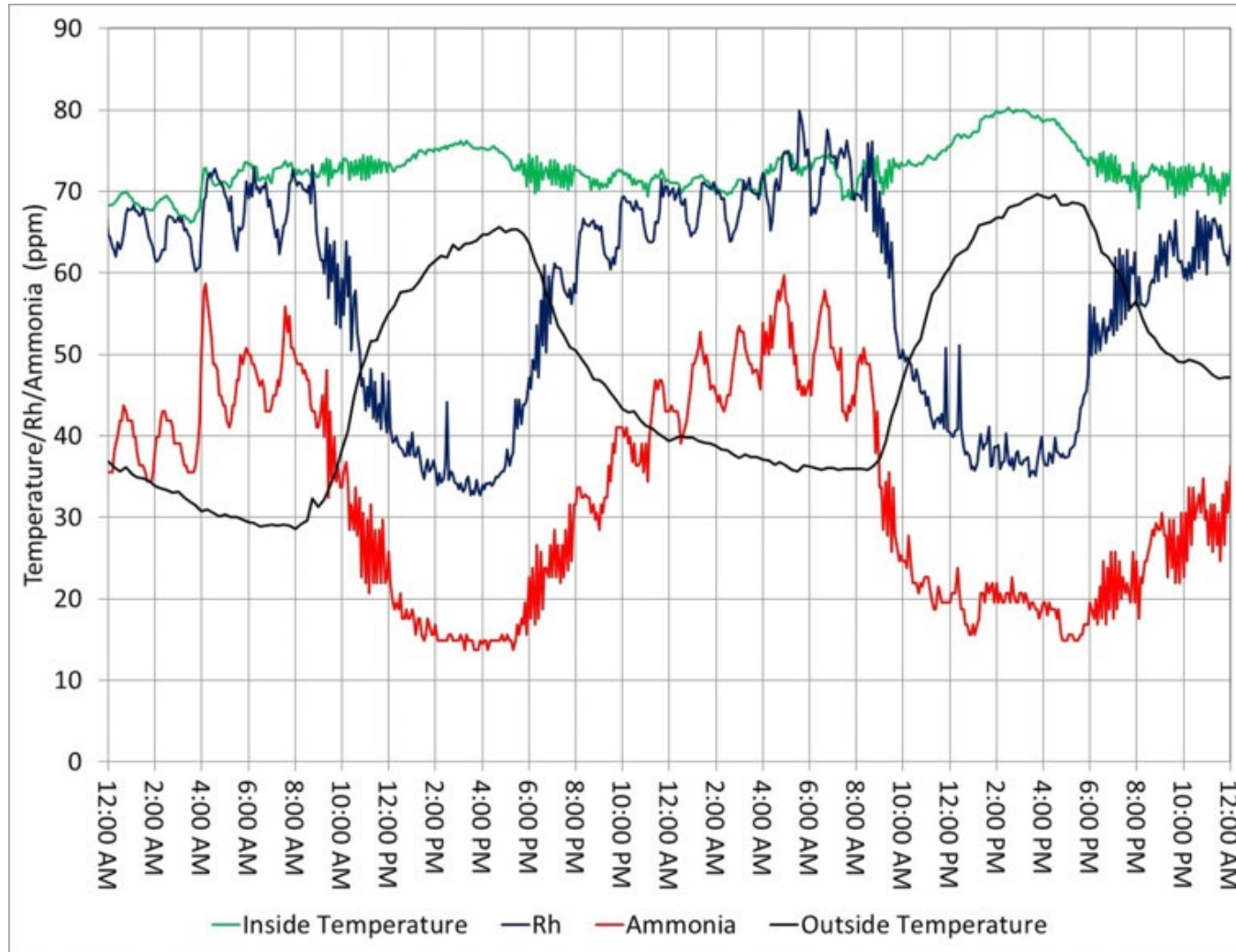


# IBV vaccination and ammonia

Does exposure to  
environmental ammonia  
affect the immune response  
to IBV vaccination?



# Ammonia levels follow Rh



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# Source of Ammonia

- Reused litter
- Fans used to control relative humidity and ammonia levels
- Standard stocking density, heat sources, feed & lighting schedule
- Ammonia level target was 30 to 60 ppm



No Ammonia



Ammonia

# Experimental design

## Expt 1

### Groups:

- -/NAM/+
- +/NAM/+
- +/Am/+

## Expt 2

### Groups:

- -/NAM/+
- -/Am/+
- +/NAM/+
- +/Am/+
- -/NAM/-

## Expt 3

### Groups:

- -/NAM/+
- -/Am/+
- +/NAM/+
- +/Am/+
- -/NAM/-

### Procedures:

- Virus detection (choanal swabs)
- Clinical signs, airsacculitis, tracheal histopathology, ciliostasis<sup>a</sup>
- ELISA (IgG – serum, IgA – tears)
- Flow cytometry (leukocytes, MHC I/II<sup>lo</sup> and MHC I/II<sup>hi</sup>, IgM<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> cells)

<sup>a</sup>5 dpc only

Day 0: Vaccinate  
IBV-Mass



28 dpv: Challenge  
IBV Mass41



Week 0 1 2 3 4 5 6



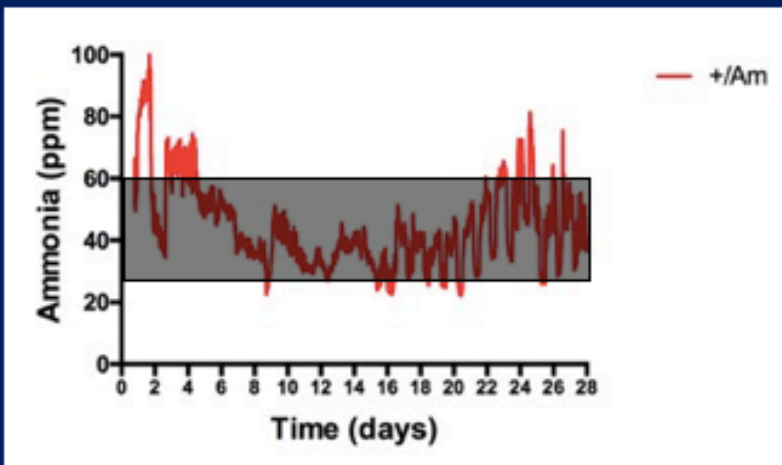
7, 10, 12, 14 dpv:  
Post-vaccination

24, 26, 28 dpv:  
Pre-challenge

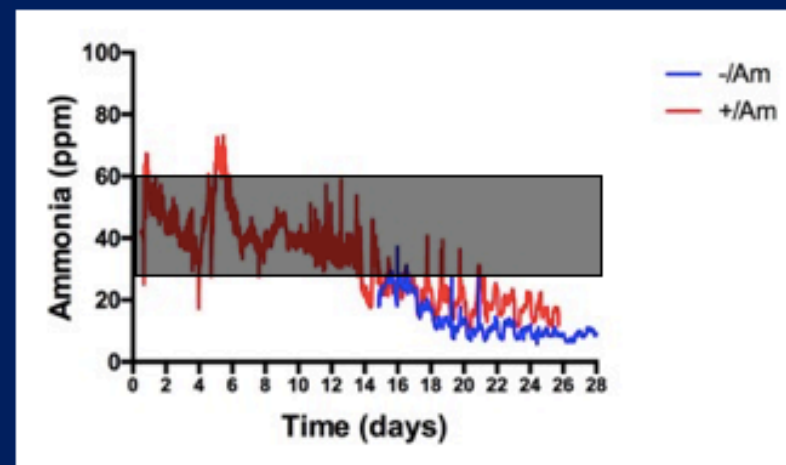
5, 10, 12, 14 dpc:  
Post-challenge

# Ammonia levels were within the target range for the first two weeks

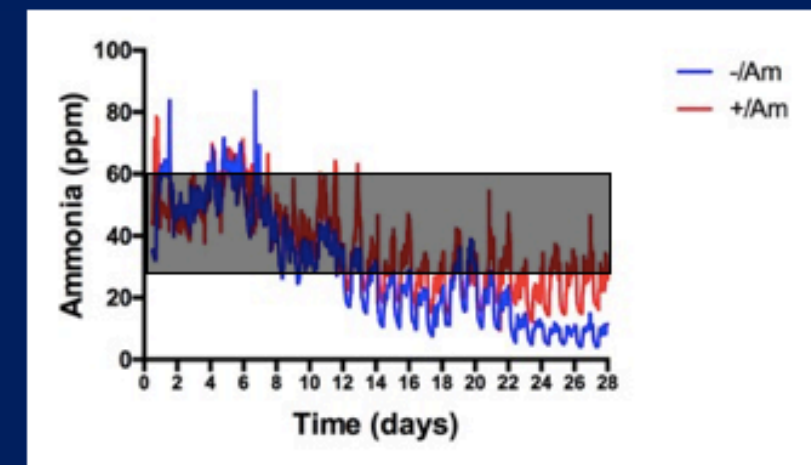
## Experiment 1



## Experiment 2



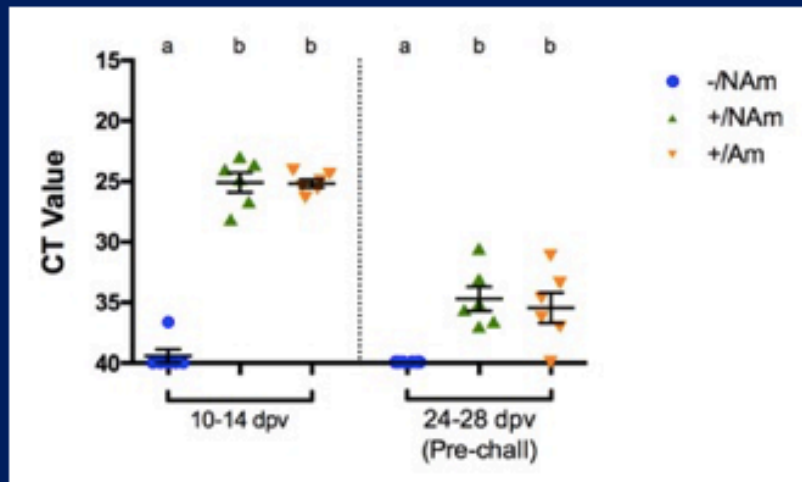
## Experiment 3



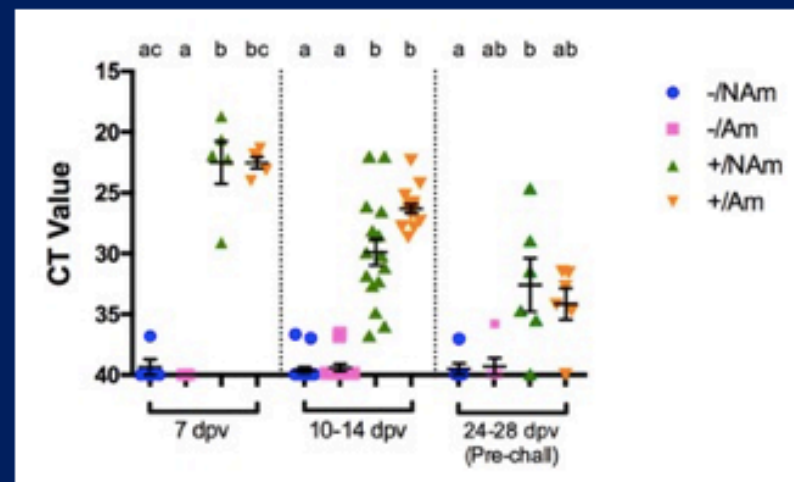


# Pre-challenge vaccine virus load was not affected by ammonia

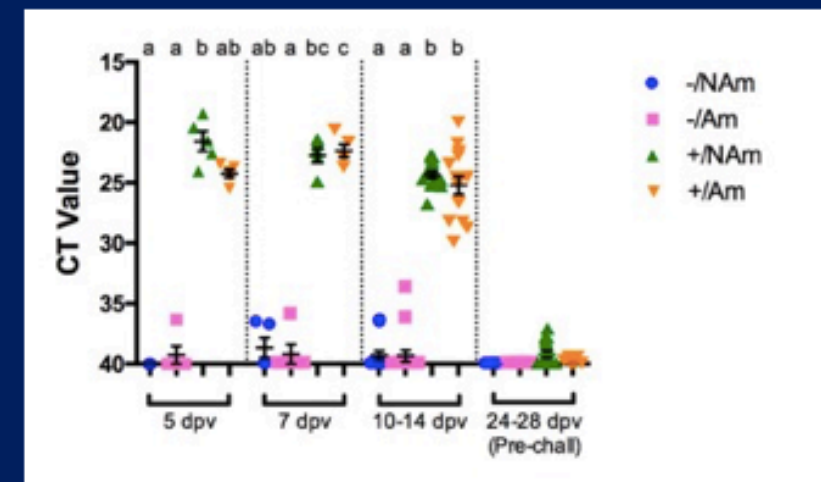
## Experiment 1



## Experiment 2



## Experiment 3



**Clinical Signs 5 days post-challenge were reduced by vaccination but not influenced by ammonia**

Treatment			Clinical Signs (Mean ± SEM <sup>a</sup> )			
Vaccine	Ammonia	Challenge	Exp 1	Exp 2	Exp 3	Average
-	-	+	2.1 ± 0.4	3.0 ± 0.0	2.3 ± 0.3	2.4 ± 0.2
-	+	+	-	3.0 ± 0.0	2.7 ± 0.2	2.9 ± 0.1
+	-	+	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
+	+	+	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
-	-	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

<sup>a</sup>SEM=standard error of the mean



**Vaccinated birds, regardless of ammonia exposure, did not show ciliostasis 5 days post-challenge**

Treatment			Ciliostasis			
Vaccine	Ammonia	Challenge	Exp 1	Exp 2	Exp 3	Average
-	-	+	0 <sup>a</sup> , 10/10 <sup>b</sup>	0, 5/5	0, 10/10	0, 25/25
-	+	+	-	0, 10/10	0, 10/10	0, 20/20
+	-	+	86.2, 1/10	90.7, 0/10	78.3, 1/9	85.1, 2/29
+	+	+	87.3, 0/10	88.3, 0/10	82.2, 1/10	85.9, 1/30
-	-	-	97.7, 0/5	96.0, 0/5	71.3, 0/5	88.3, 0/15

## Tracheal histopathology scores were reduced by vaccination but not affected by ammonia

Treatment			Histopathology Score (Mean $\pm$ SEM <sup>a</sup> )			
Vaccine	Ammonia	Challenge	Exp 1	Exp 2	Exp 3	Average
-	-	+	9.8 $\pm$ 0.1	9.5 $\pm$ 0.0	9.6 $\pm$ 0.2	9.6 $\pm$ 0.1 <sup>a</sup>
-	+	+	-	9.6 $\pm$ 0.1	9.6 $\pm$ 0.1	9.6 $\pm$ 0.0 <sup>a</sup>
+	-	+	6.4 $\pm$ 0.4	6.1 $\pm$ 0.1	6.6 $\pm$ 0.2	6.3 $\pm$ 0.2 <sup>b</sup>
+	+	+	5.1 $\pm$ 0.5	6.2 $\pm$ 0.3	6.5 $\pm$ 0.6	6.0 $\pm$ 0.3 <sup>b</sup>
-	-	-	6.0 $\pm$ 0.4	5.1 $\pm$ 0.6	4.7 $\pm$ 0.5	4.9 $\pm$ 0.3 <sup>c</sup>

<sup>a</sup>SEM=standard error of the mean

## Nonvaccinated birds stressed with ammonia experienced a higher incidence of airsacculitis 5 days post-challenge

Treatment			Airsacculitis			
Vaccine	Ammonia	Challenge	Exp 1	Exp 2	Exp 3	Total
-	-	+	5/10	2/5	3/10	10/25
-	+	+	-	9/10	7/10	17/20
+	-	+	0/10	0/10	0/9	0/29
+	+	+	0/10	0/10	0/10	0/30
-	-	-	0/5	0/5	0/5	0/15

<sup>a</sup>SEM=standard error of the mean

## **Post-vaccination immune trends**

No ammonia-related effect

Vaccine-related effects:

IBV-specific serum IgG and tear IgA responses weak to nonexistent 10-14 dpv

Harderian Gland

- Increased % of leukocytes
- Increased % of leukocytes with high MHC II expression
- Increased % of IgM<sup>+</sup> cells
- Increased % of CD4<sup>+</sup> and CD8<sup>+</sup> cells, decreased CD4:CD8 ratio

## Post-challenge immune trends

No ammonia-related effects

### Vaccine-related effects in harderian gland

5 dpc

- Increased % of leukocytes in nonvaccinated, challenged birds
- Increased % of leukocytes with high MHC II expression
- Increased % of IgM<sup>+</sup> cells in nonvaccinated, challenged birds

10-14 dpc

- Increased % of CD4<sup>+</sup> and CD8<sup>+</sup> cells in nonvaccinated, challenged birds

10-14 dpc

- Increased IBV-specific serum IgG titers in all challenged birds, but especially in nonvaccinated, challenged birds
- Increased IBV-specific IgA titers in tears in nonvaccinated, challenged birds

# Discussion

Ammonia levels between 30 and 60 ppm did not affect immunity to IBV following vaccination (higher levels might have an affect)

Ammonia, combined with incomplete vaccine coverage, may exacerbate respiratory disease

Maximizing vaccine coverage and managing ammonia levels are strategies to decrease airsacculitis and reduce antibiotic usage



# Acknowledgments

Emily Aston  
Brian Jordan  
Brian Fairchild  
Robert Gogal, Jr.  
Maricarmen Garcia  
David Hurley  
Debbie Hilt  
Sunny Cheng  
Laura Tensa  
Grace Albanese  
Jongseo “Johnny” Mo  
Julia McElreath

