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Systems-Based Approach to *Salmonella* Ecology in Pastured Poultry Systems

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What is Pastured Poultry?

Conventional chicken operations

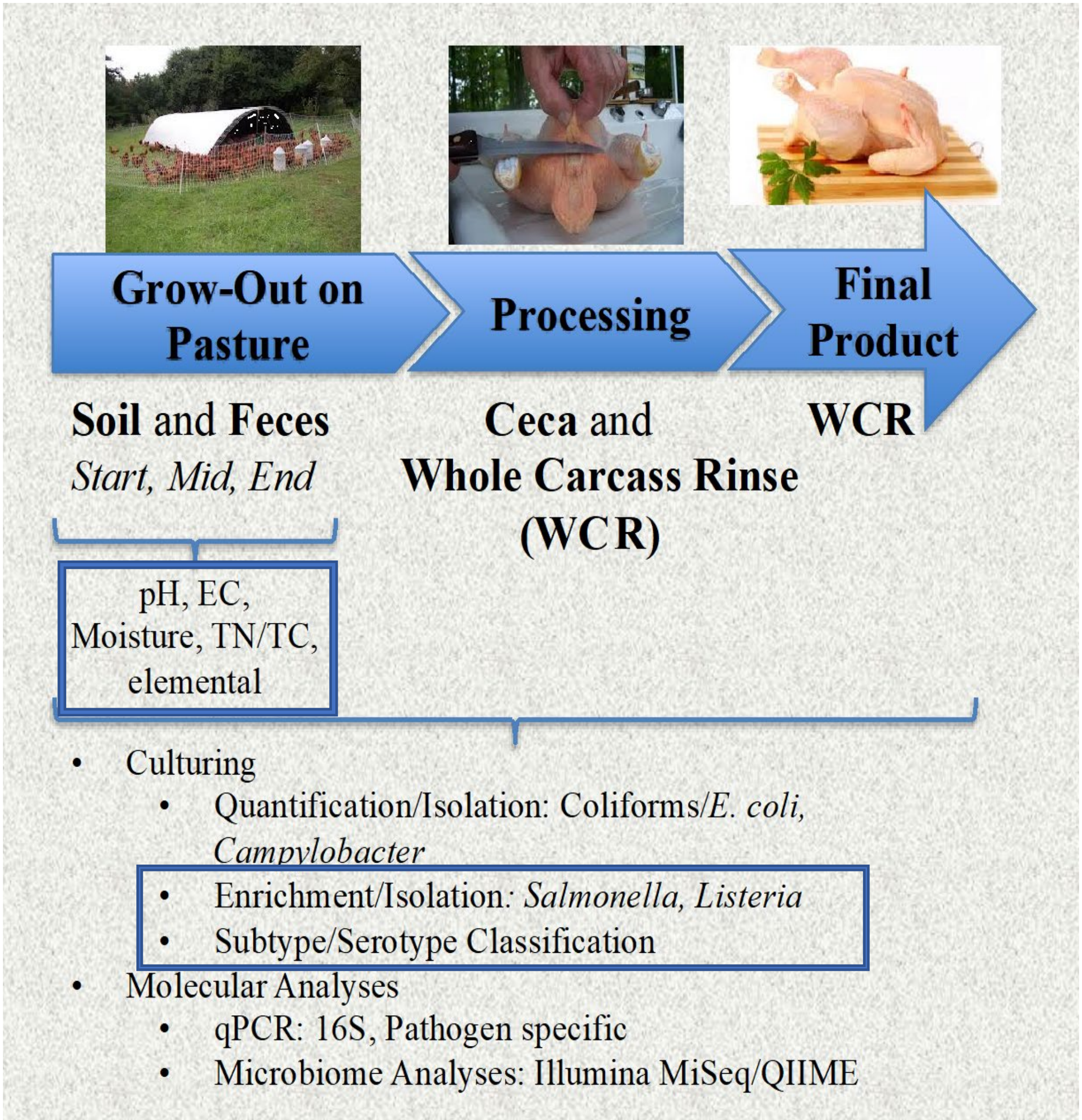
Pasture-raised chicken operations

Why Focus on Pastured Poultry Producers?

- Lots of interest in locally-grown, "all natural" agricultural products
  - *To feed the globe, we will need to eat more locally*
  - How a lot of the world produces their food
- There is a definite shift in the poultry marketplace towards antibiotic-free, no antibiotics ever, "all-natural", free range products, which are all versions of what small producers are already doing on their farms
- Access to farms is key to a lot of applied research studies, and in my experience, small producers have provided greater access than conventional operations
  - More flexibility in research goals too

Pastured Poultry Project

- 2014-2017: Following 42 flocks from 11 farms, looking at pasture feces/soil during grow-out, processing samples, and final product samples
    - Pre-Harvest data collected;
        - Management data (questionnaires)
        - Physiochemical data (pH, EC, moisture, nutrients/elements)
        - Meteorological data (temperature, rainfall, wind, humidity)
        - Biological data (*Salmonella, Campy, Listeria,* Microbiome)



Rothrock et al (2021)
https://doi.org/10.3389/fanim.2021.761930

Salmonella prevalence based on sample type or farm of origin

	No. of + samples	No. of - samples	Prevalence[1]
		Sample Type	
Feces	124	691	0.152[B]
Soil	94	721	0.115[B]
Ceca	27	183	0.129[B]
Processing WCR	67	168	0.285[A]
Final Product WCR	41	189	0.178[AB]
		Farm	
A	107	398	0.212[AB]
B	35	220	0.137[A]
C	3	42	0.067
D	1	44	0.022
E	45	241	0.157[A]
H	24	86	0.218[AB]
I	101	289	0.259[A]
J	0	110	0.000
K	8	272	0.029[C]
L	0	150	0.000
M	29	101	0.223[AB]

[1] Superscript letters indicated significantly different prevalence values based on ANOVA analyses using the Tukey's post-test at a significance level of $p < 0.05$. For the Farm data, only farms that were followed over multiple years were included in the statistical analyses.

In no sample were all 5 subsamples positive for *Salmonella*

Pre-Harvest:

~15% feces, ~12% soil

Post-Harvest:

~13% ceca, ~28% Proc WCR

~18% Final Prod. WCR

High variation between farms, with multiple farms showing an absence of *Salmonella* for different sample types

But who are the main *Salmonella* players, and how do they relate to food safety targets?

Rothrock et al (2021)
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Salmonella serotype diversity

	No. of *Salmonella* isolates	Serotype Prevalence[1]	CDC top 32[2]
Kentucky	245	0.7270	No
Indiana	32	0.0950	No
Infantis	20	0.0593	Yes
Enteritidis	9	0.0267	Yes
Seftenberg	7	0.0208	Yes
Braenderup/Cholareasuis	6	0.0178	Yes
Meleagridis	5	0.0148	No
Muenchen	5	0.0148	Yes
1,4,[5],12:I:-	2	0.0059	Yes
Heidelberg	1	0.0030	Yes
Javiana	1	0.0030	Yes
Mbandaka/Typhimurium	1	0.0030	Yes
Orion	1	0.0030	No
Schwarzengrund	1	0.0030	Yes
Unclassified	1	0.0030	NA

[1] Calculated based on the No. of isolates for a given serotype divided by the total number of *Salmonella* isolates (337).
[2] Based on the CDC Atlas of Salmonella in the United States, 1968-2011.

Kentucky, by far, was the most prevalent serotype

Only ~16% (53/337) of the isolates belonged to serotypes that are important to human health!

Was this diversity sample type or farm dependent?

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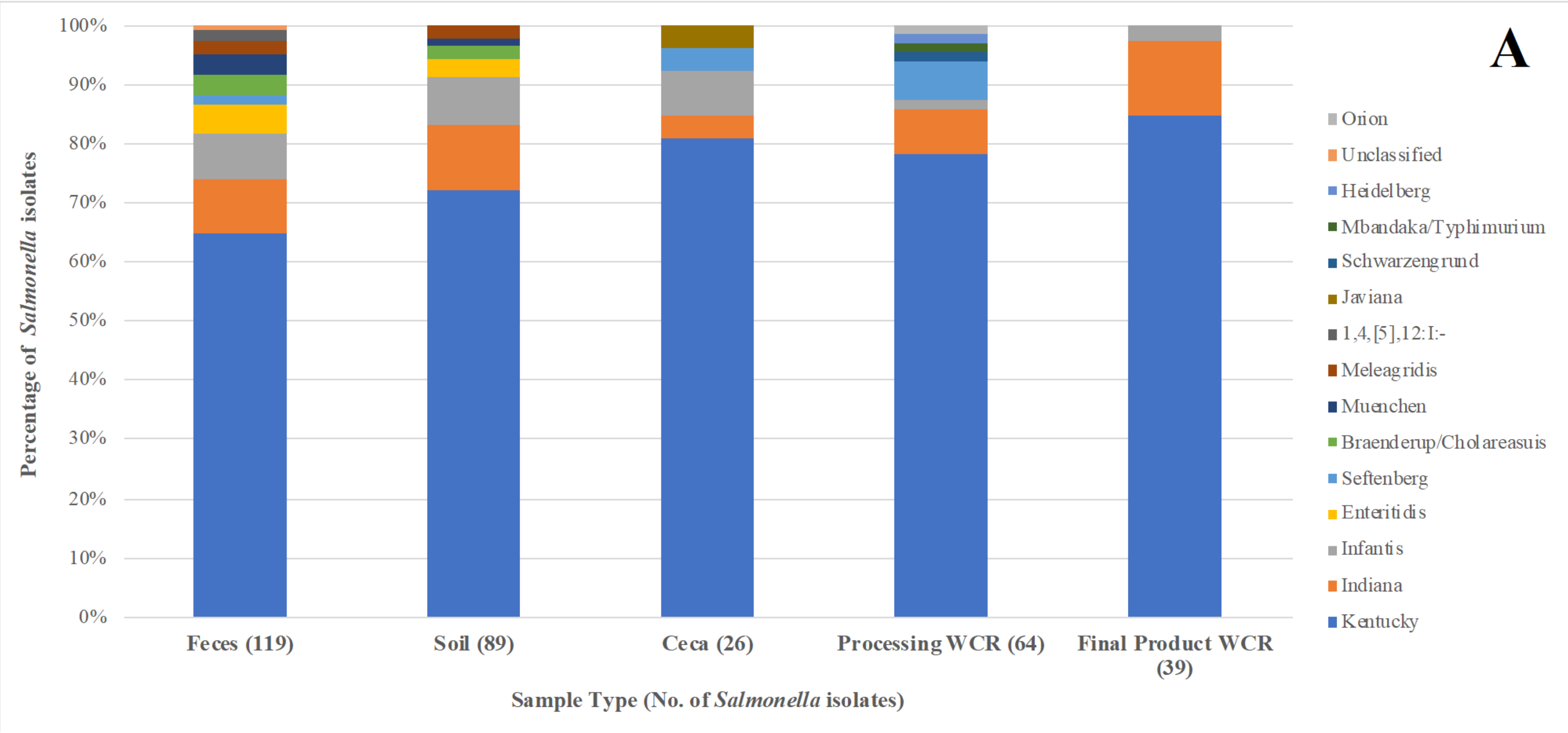

Diversity profiles were relatively stable over the different sample types

Rothrock et al (2021)
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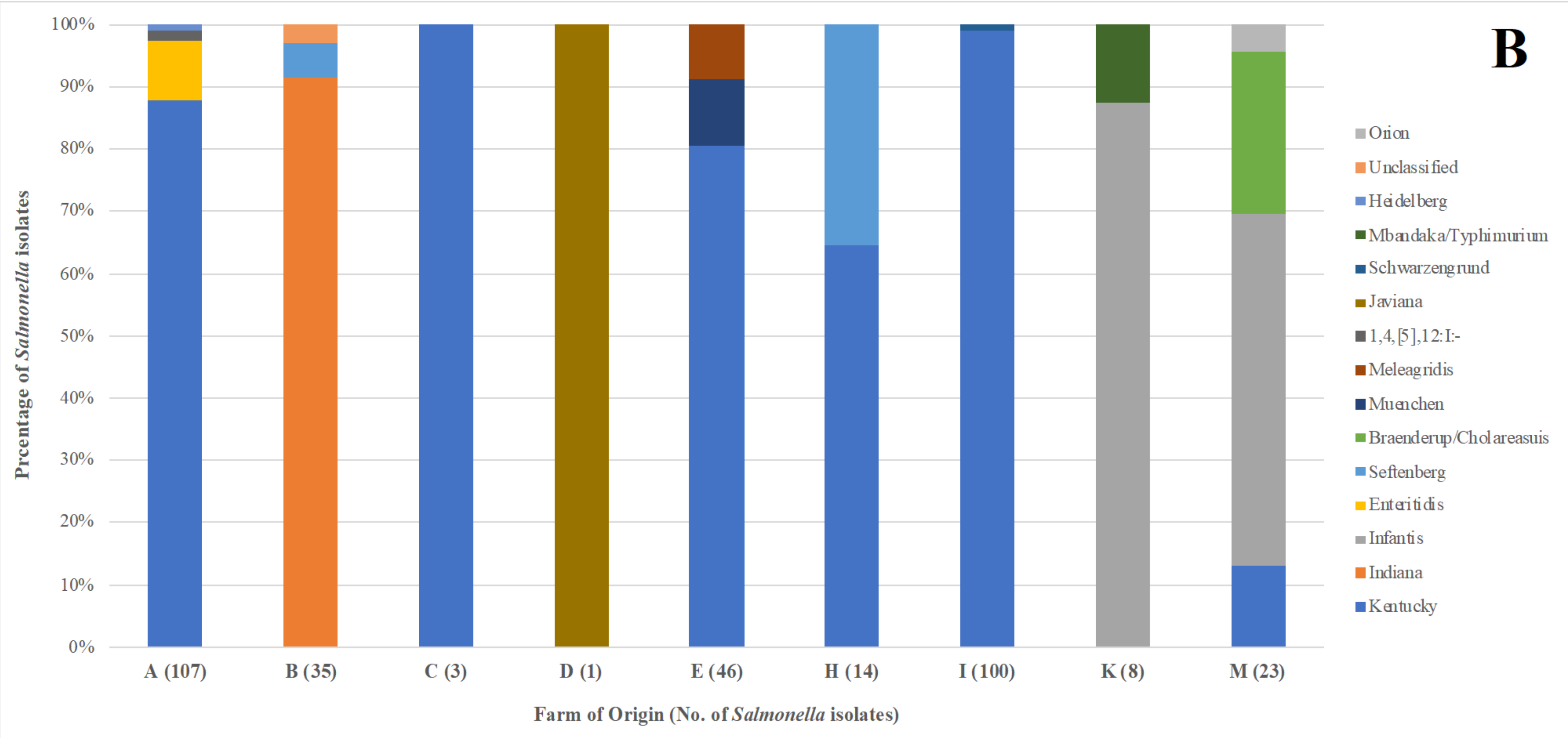

Diversity profiles appear to be farm-dependent

Rothrock et al (2021)
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Multidrug resistance among *Salmonella* isolated from this study[1]

	No. of isolates	MDR isolates	MDR Rate
		Sample Type	
Feces	119	30	0.252
Soil	89	34	0.382
Ceca	26	2	0.077
Processing WCR	64	13	0.203
Final Product WCR	39	13	0.333
		Farm	
A	107	80	0.748
B	35	1	0.029
C	3	3	1.000
D	1	0	0.000
E	46	0	0.000
H	14	8	0.571
I	100	0	0.000
J	0	0	0.000
K	8	0	0.000
L	0	0	0.000
M	23	1	0.043

[1] Isolates were considered multidrug resistant (MDR) if the expressed resistance to ≥3 antibiotics on the gram negative NARMS protocol and breakpoints.

In the absence of antibiotic use on farm, ~27% (92/337) of the isolates were considered MDR

Aside from ceca, MDR rates were similar among sample types, but large differences were observed based on farm of origin.

Some farms even exhibited unique AR profiles

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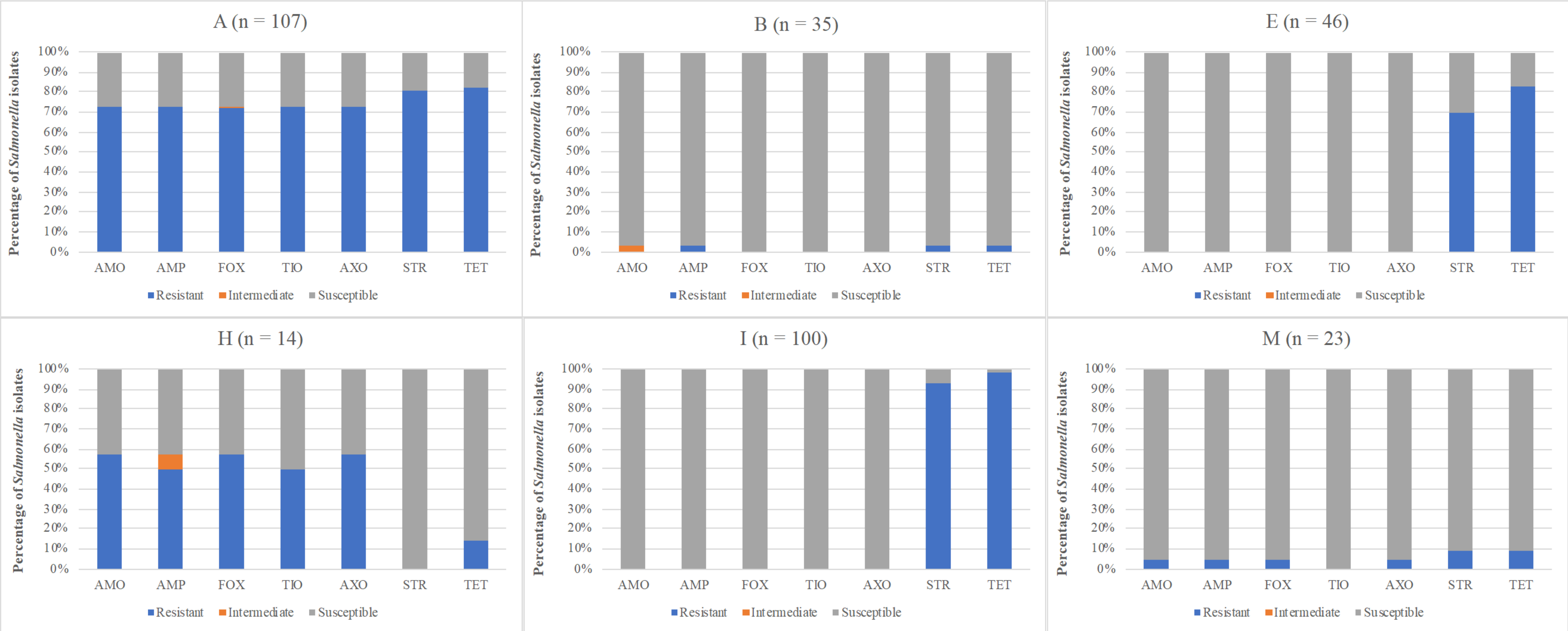


Indicative of potential carriage and survival of *bla*$_{CMY-2}$ gene on a plasmid within the farm's infrastructure

Dr. Mishra
UGA

Predictive Modeling of *Salmonella* Prevalence

Management

Brood Feed
Pasture Feed
Years Farming
Water Source
Other Animals Reared
Flock Age
Rearing Season

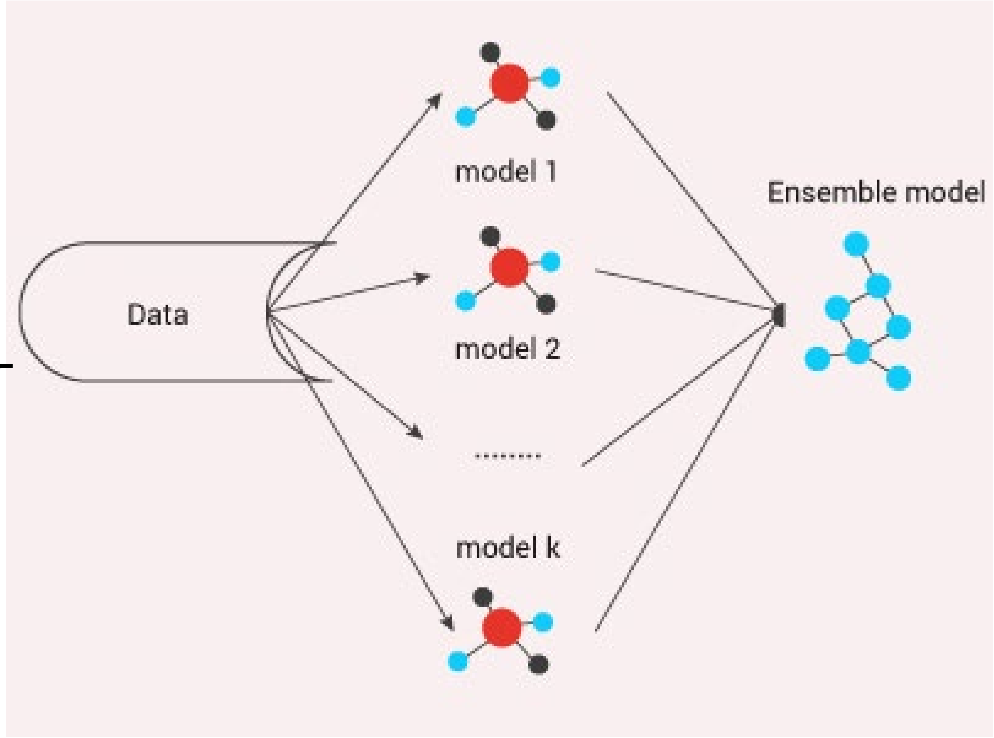
Meteorological

Avg Max Temp
Avg Mean Temp
Avg Min Temp
Avg Humidity
Avg Wind Speed
Avg Wind Gust Speed
Rainfall

Samples

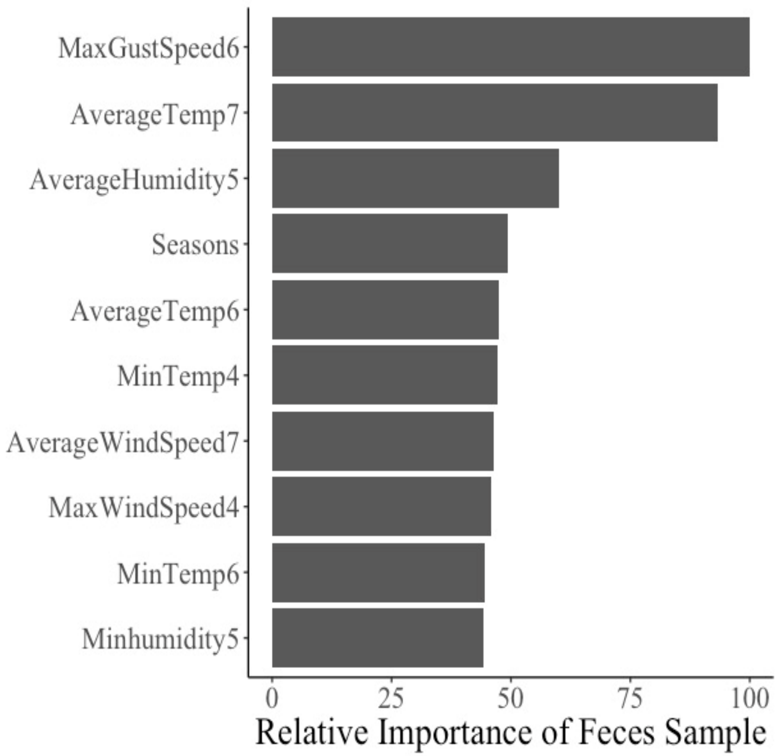
Feces (Pre-Harvest) WCR (Post-Harvest)

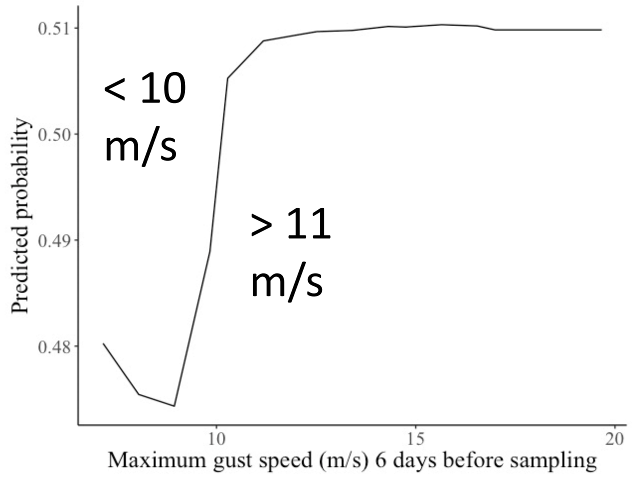
Random Forest Modeling

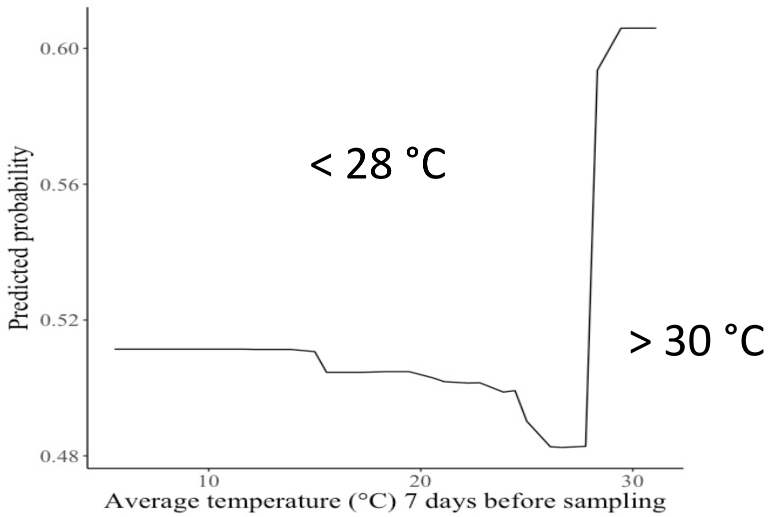


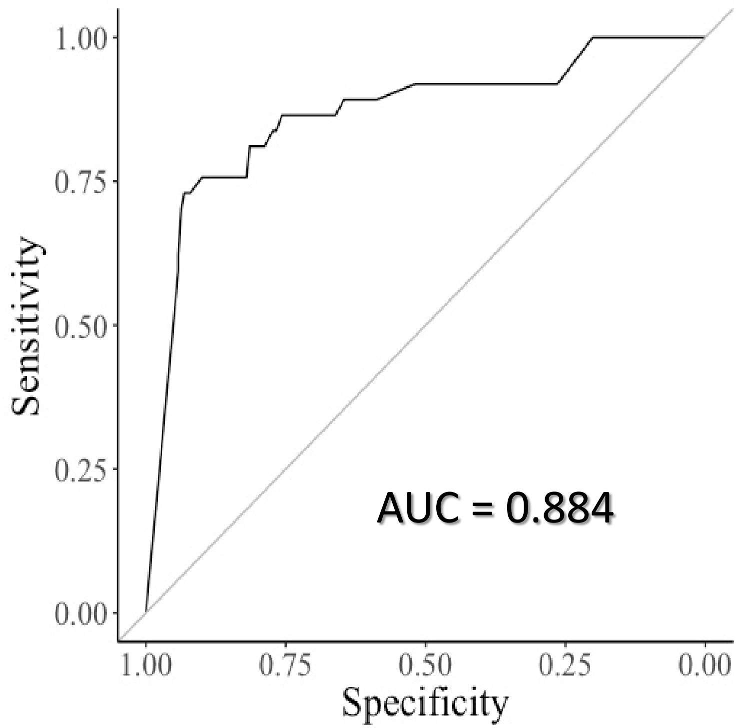
Hwang et al (2020)
https://doi.org/10.1016/j.scitotenv.2019.136359

Meteorological Factors



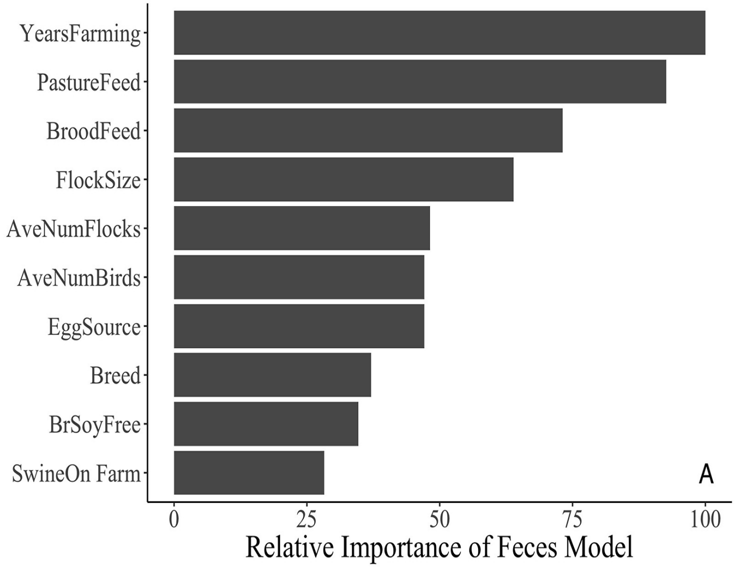


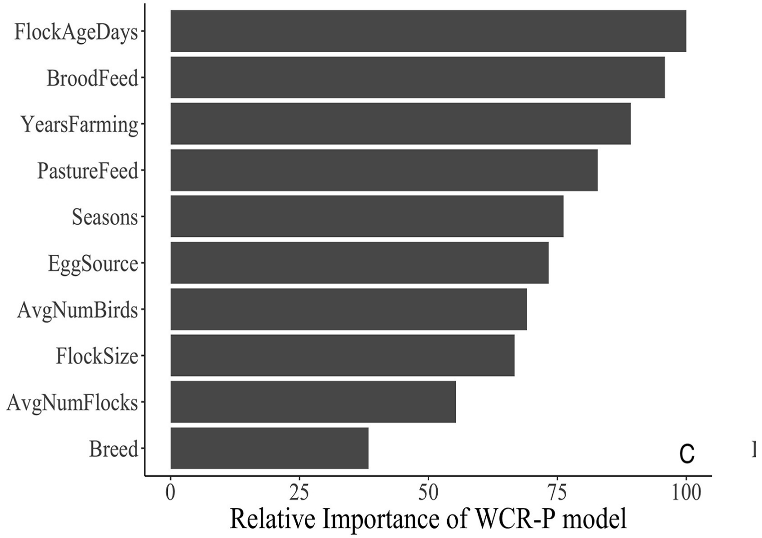




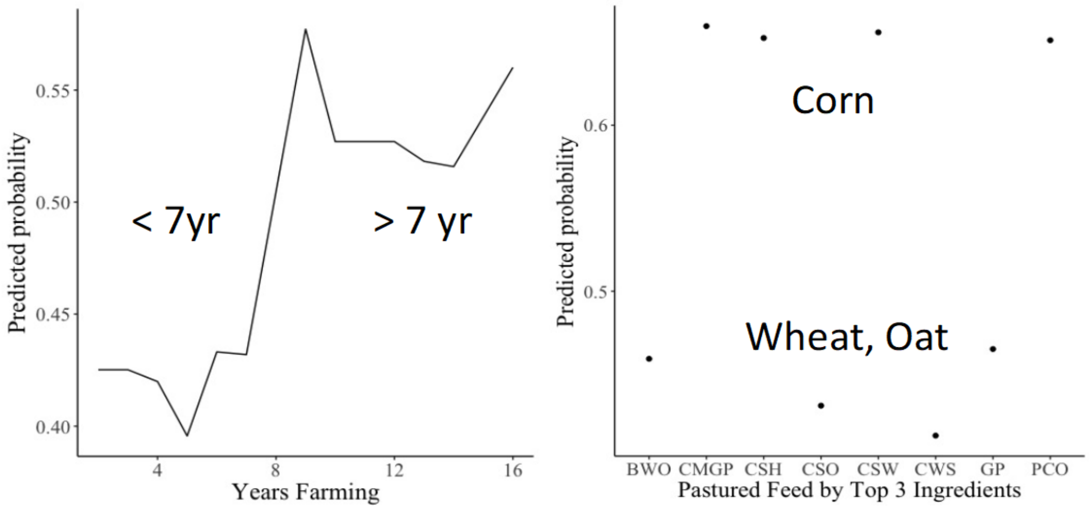
Hwang et al (2020)
https://doi.org/10.1016/j.lwt.2020.109423

Management Factors



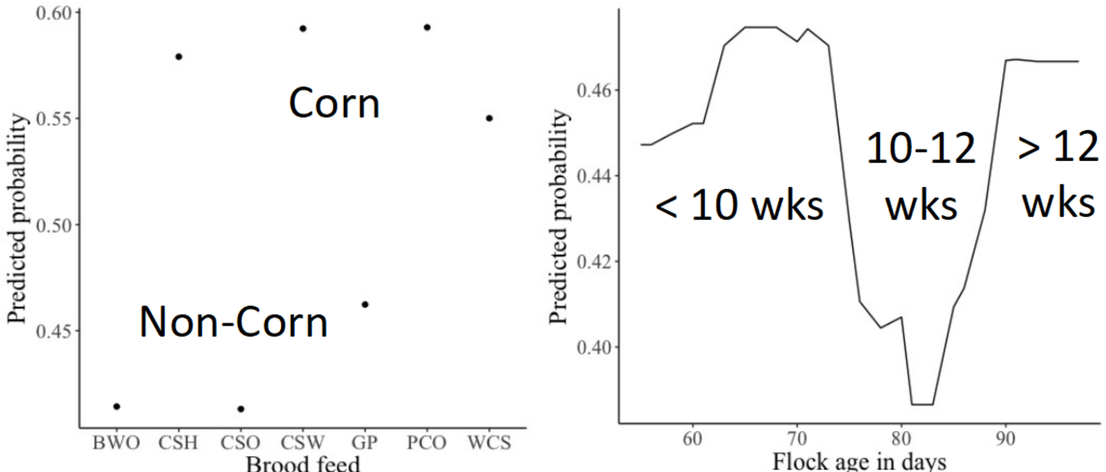


Salmonella Pre-Harvest Management



AUC = 0.93

Salmonella Post-Harvest Management



AUC = 0.83

Lets Go “Deeper” - Ensemble approach

- Predictions based on single model
  - Problem: Feature importance varies with the model
- Ensemble Approach to increase confidence in prediction
  - 5 different models with same dataset
    - Random Forest (RT)
    - XGBoost (XG)
    - Three *deep learning* approaches
      - Multi-layer perceptron (MLP)
      - AutoEncoder (ENC)
      - Generative Adversarial Network (GAN)

Drs. Nanduri & Ramkumar
MSU

Model Input Variables

1	Seasons
2	AvgNumBirds
3	AvgNumFlocks
4	YearsFarming
5	EggSource
6	BroodBedding
7	BroodFeed
8	BrGMOFree
9	BrSoyFree
10	BroodCleanFrequency
11	AvgAgeToPasture
12	PastureHousing
13	FreqHousingMove
14	AlwaysNewPasture
15	PastureFeed
16	PaGMOFree
17	PaSoyFree
18	LayersOnFarm
19	CattleOnFarm
20	SwineOnFarm
21	GoatsOnFarm
22	SheepOnFarm
23	WaterSource
24	FreqBirdHandling
25	AnyABXUse
26	FlockAgeDays
27	Breed
28	FlockSize
29	AnimalSource

Previous Predictive Modeling

1	pH
2	EC
3	Moisture
4	TotalC
5	TotalN
6	CNRatio
7	Ca
8	Cd
9	Cr
10	Cu
11	Fe
12	K
13	Mg
14	Mn
15	Mo
16	Na
17	Ni
18	P
19	Pb
20	Zn

Physiochemical analysis

Critical Farm Variables

Sample Type	Model	Electrical Conductivity (EC)	Magnesium(Mg)	Flock Size	Sodium (Na)	Zinc (Zn)	Potassium(K)
Feces	RT	1	5				8
	XG						
	MLP		3	1		13	
	ENC		3				2
	GAN		4	1			3
Soil	RT			2	6	8	
	XG						
	MLP	3	8	1	4	9	2
	ENC	2			1	5	
	GAN	4	2	1	3	6	

Recommendations to Reduce *Salmonella* Prevalence

Farm Variable	Feces	Soil
K	> 5000 ppm	
GMO Free Pasture/Brood feed	No	
Cu	< 10 ppm	
Ca	< 10000 ppm	
Average Number of birds		> 1000 ppm
Soy free Brood feed		No
EC		> 100 mS/m
Flock size		>500
Na, Zn		> 20 ppm
Years of farming		< 3

Evaluation: Production Scale

- Flock size - a confounding farm variable
- Evaluations based on production scale
  - Professional farming - Greater than 3 flocks
  - Hobby farming - fewer flocks
- Recommendations based on fecal and soil models

Recommendations to Reduce *Salmonella* Prevalence

Feces

Farm Variable	Professional	Hobby
Mn	< 100 ppm	< 100 ppm or > 150 ppm
K	5000 – 6000 ppm	< 5000 ppm
Pb	< 14 ppm	< 5 ppm
CN Ratio		< 15
Mg	2000 – 3500 ppm	
Na	< 1000 ppm	
EC	3000 – 4000 ppm	
Flock age (days)	< 30	
Soy free brood feed	Yes	

Soil

Farm Variable	Professional	Hobby
P	< 600 ppm	
Ca	< 1000 ppm	
Years farming	> 8	
Soy free brood feed	Yes	
Mn		40 – 100 ppm
Flock size		> 25
Na		> 10 ppm

Future Directions

- Use microbiome data as the input data for *Salmonella* to test not only meteorological, physicochemical, and management variables, but also biological variables
    - Ex: Relationship of *Salmonella* to pre-/pro-biotic taxa (e.g. *Lactobacillus, Streptococcus*) and how these relationships change based on management variables
- Further explore the link between pre-harvest and processing variables and final product *Salmonella* prevalence using deep learning methodologies

Expand Beyond Pastured Poultry Systems

- This work highlights the potential for these systems-based approaches in conventional poultry systems, especially as they begin to adopt some of these systems with broilers have greater access to land outside of the houses
- The more datapoints for each type of metadata you have, the better the models become, and FSIS has access to a lot of data (especially from post-harvest environments).
- Potential to provide actionable data and recommendations to stakeholders to enhance poultry food safety

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Acknowledgements

Dr. Aude Locatelli
Dr. Jean Guard
Dr. Ade Oladeinde
Dr. Richard Gast
Latoya Wiggins
Laura Lee-Rutherford
Kim Ingram
Dr. Kelli Hiett
Katelyn Griffin
Cheryl Gresham
Manju Amin
Sydney Graham (UW)

UGA MPH Interns
Ashland Roquemore
Destin Miller
Joseph Fujikawa
Dr. Abhinav Mishra
Chase Golden
Daizy Hwang
Xinran Xu

Dr. Steven Ricke
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Dr. Kristina Feye

Dr. Bindu Nanduri
Dr. Mahalingam Ramkumar
Dr. Moses Ayoola
Dr. Nisha Pillai