Royal Veterinary College
University of London

Chicken caecal enterotypes are determined by genetic and environmental factors and influence susceptibility to *Campylobacter* and other pathogens

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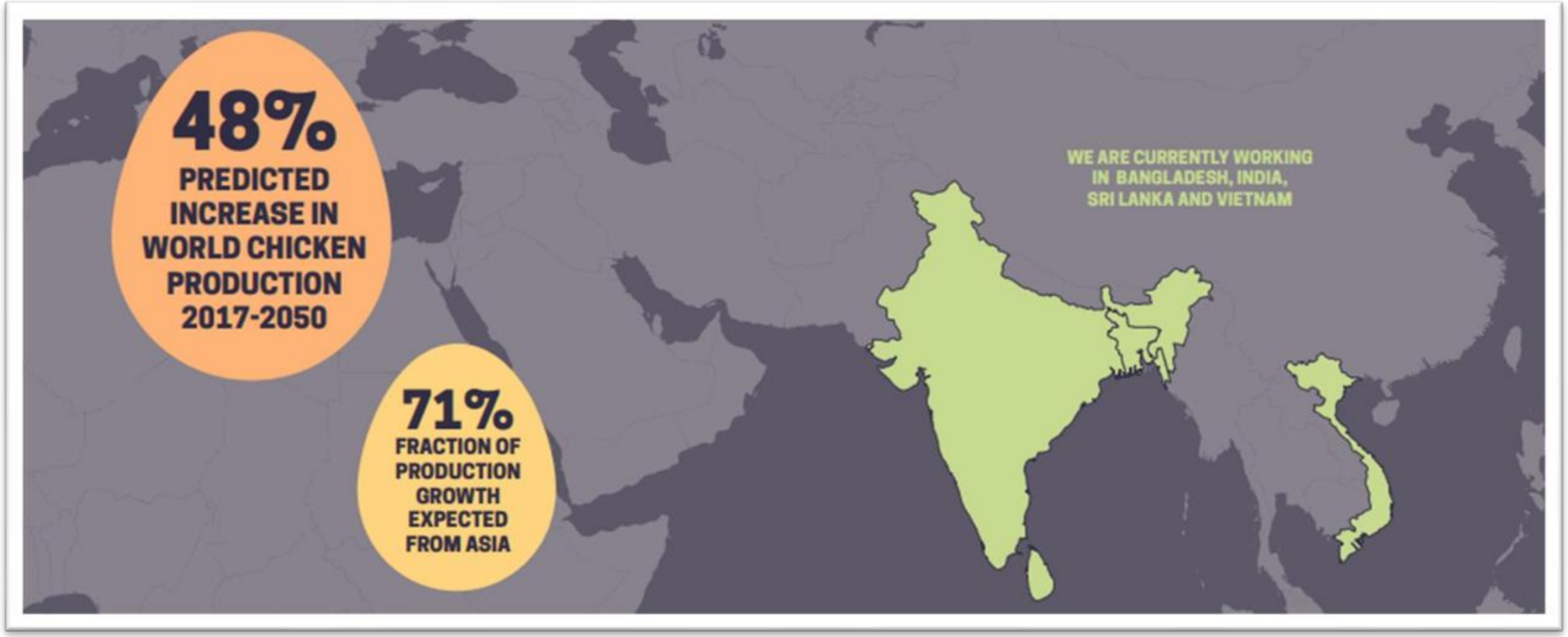
UKRI
Biotechnology and Biological Sciences Research Council

Prof Damer Blake,
Prof Fiona Tomely

Manipulating Chicken Microbiomes for good

Poultry farming and sustainability

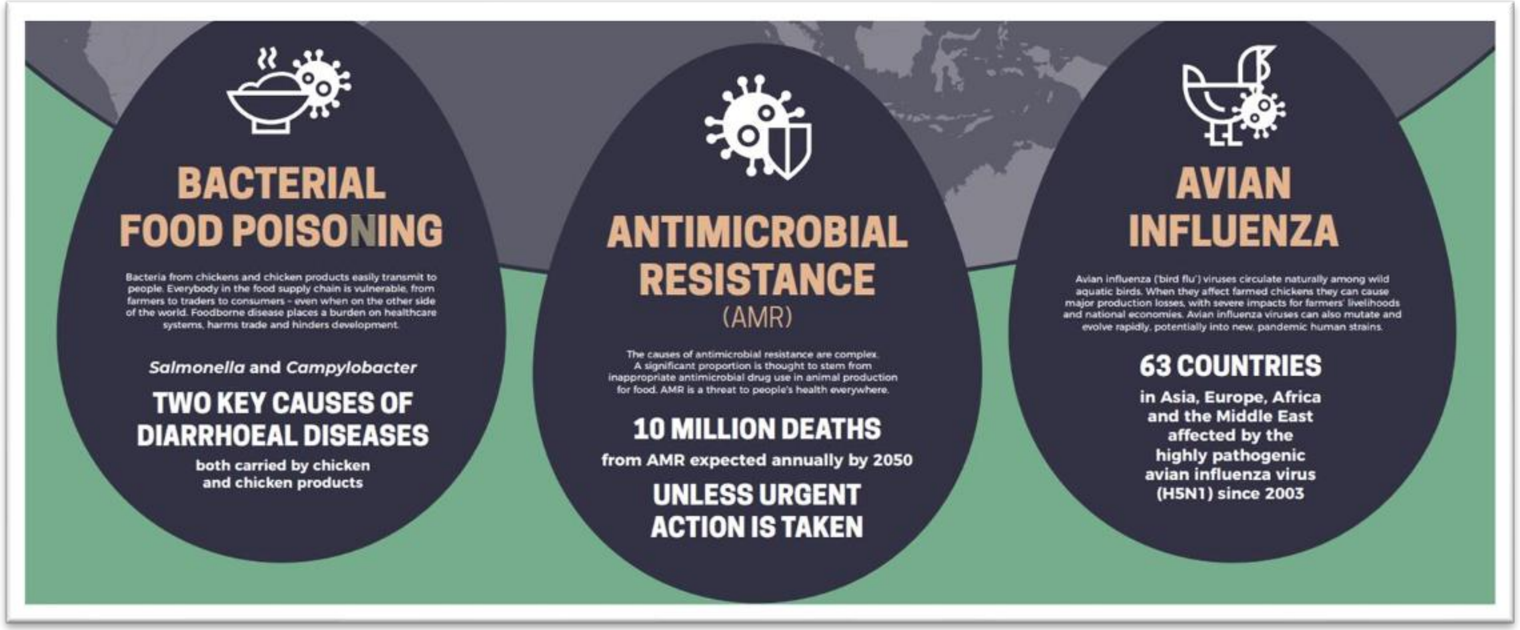
- Human population increasing in South Asia
- High quality protein sources needed
- Chickens a great source: efficient feed conversion rate means economical, good for environment



RVC

Poultry farming and pathogens

- Novel zoonotic diseases
- Known human pathogens: bacterial food poisoning
  - *Campylobacter jejuni*
  - *Salmonella*
  - *Escherichia coli*
- Antimicrobial resistance (AMR)



Antimicrobial resistance is a sustainability issue

- Sustainability means leaving the planet and environment in the same condition, or better for future generations.

- Therefore,

- if future generations inherit the AMR problem we created, we have failed.

Balancing pathogens, productivity and AMR

- Pathogens
  - *Campylobacter jejuni*
  - *Salmonella*
  - *Escherichia coli*
- Antimicrobial resistance

+ better food conversion, therefore lower carbon costs

Antibiotics

Balance risk

RVC

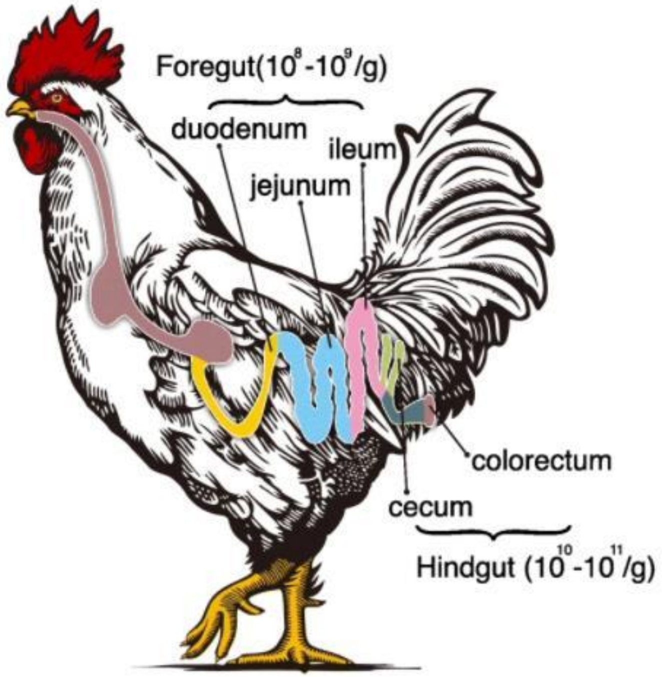
AMR is not a risk we can afford to take

- We MUST reduce our reliance on antibiotics
- How can we still:
  - reduce pathogens in poultry – (and humans)
  - get efficient food conversion? – (less feed = less land for feed, less carbon)

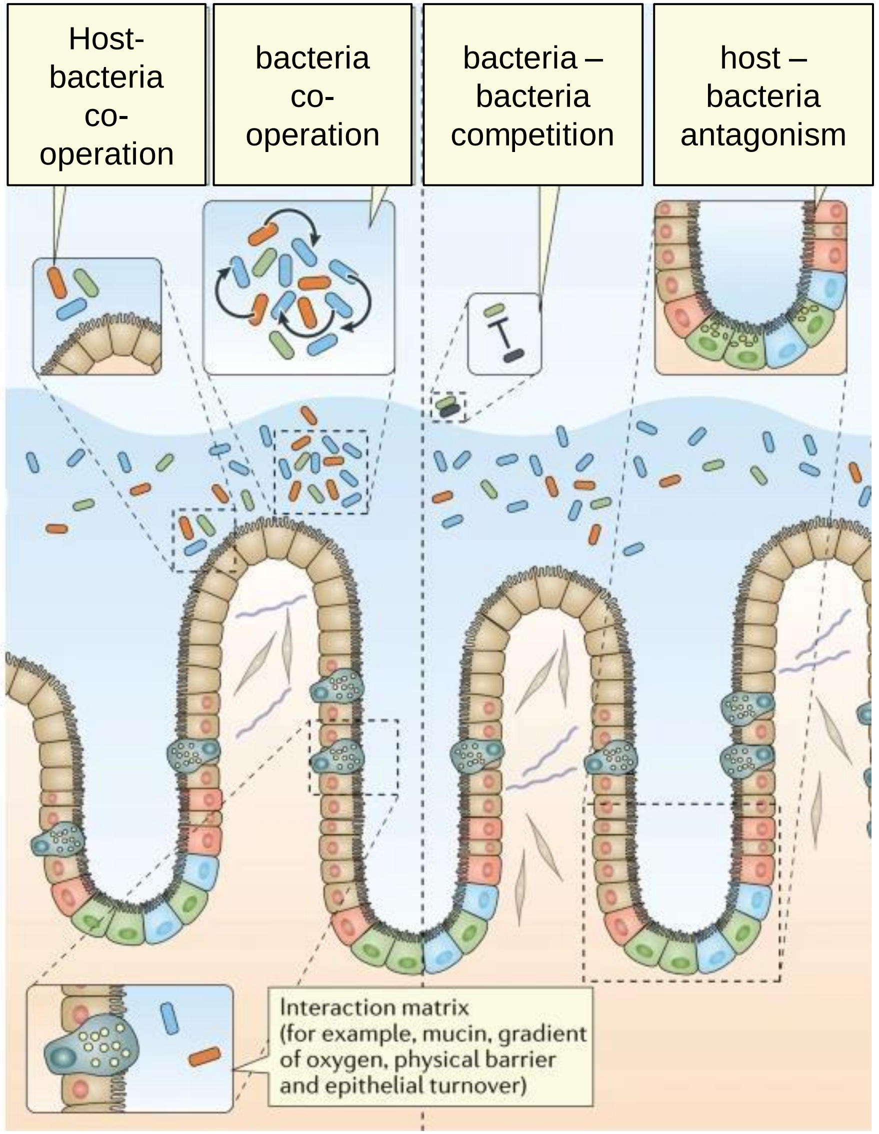
RVC

What is the microbiome?

- The microbiome encompasses all microorganisms living within an environment
- Different types and quantities of bacteria living within different sites of a chicken
- Caecal microbiome is the most abundant and diverse



The microbiome is a system in dynamic equilibrium



Sommer, F., Anderson, J., Bharti, R. et al. The resilience of the intestinal microbiota influences health and disease. Nat Rev Microbiol 15, 630–638 (2017).

The effect of the caecal microbiome on health

microorganisms MDPI

Review

Microbiota, Gut Health and Chicken Productivity: What Is the Connection?

Comparative Study > Animal. 2016 Aug;10(8):1271-9. doi: 10.1017/S1751731116000173. Epub 2016 Mar 3.

Influence of different prebiotics and mode of their administration on broiler chicken performance

M Bednarczyk [1], K Stadnicka [1], I Kozłowska [1], C Abiuso [2], S Tavaniello [2], A Dankowiakowska [1], A Sławińska [1], G Maiorano [2]

Review Article | Published: 08 February 2019

Chicken Gut Microbiome and Human Health: Past Scenarios, Current Perspectives, and Futuristic Applications

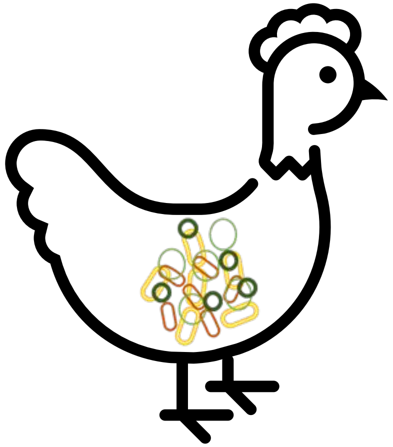
Utkarsh Sood, Vipin Gupta, Roshan Kumar, Sukanya Lal, Derek Fawcett, Supriya Rattan, Gerrard Eddy Jai Poinern & Rup Lal

Investigating the Association Between the Caecal Microbiomes of Broilers and *Campylobacter* Burden

Ioannis Sakaridis[1*], Richard J. Ellis[2], Shaun A. Cawthraw[2], Arnoud H. M. van Vliet[1], Dov J. Stekel[3], Johanna Penell[4], Mark Chambers[1,2], Roberto M. La Ragione[1] and Alasdair J. Cook[1*]

Application of Methods for Identifying Broiler Chicken Gut Bacterial Species Linked with Increased Energy Metabolism

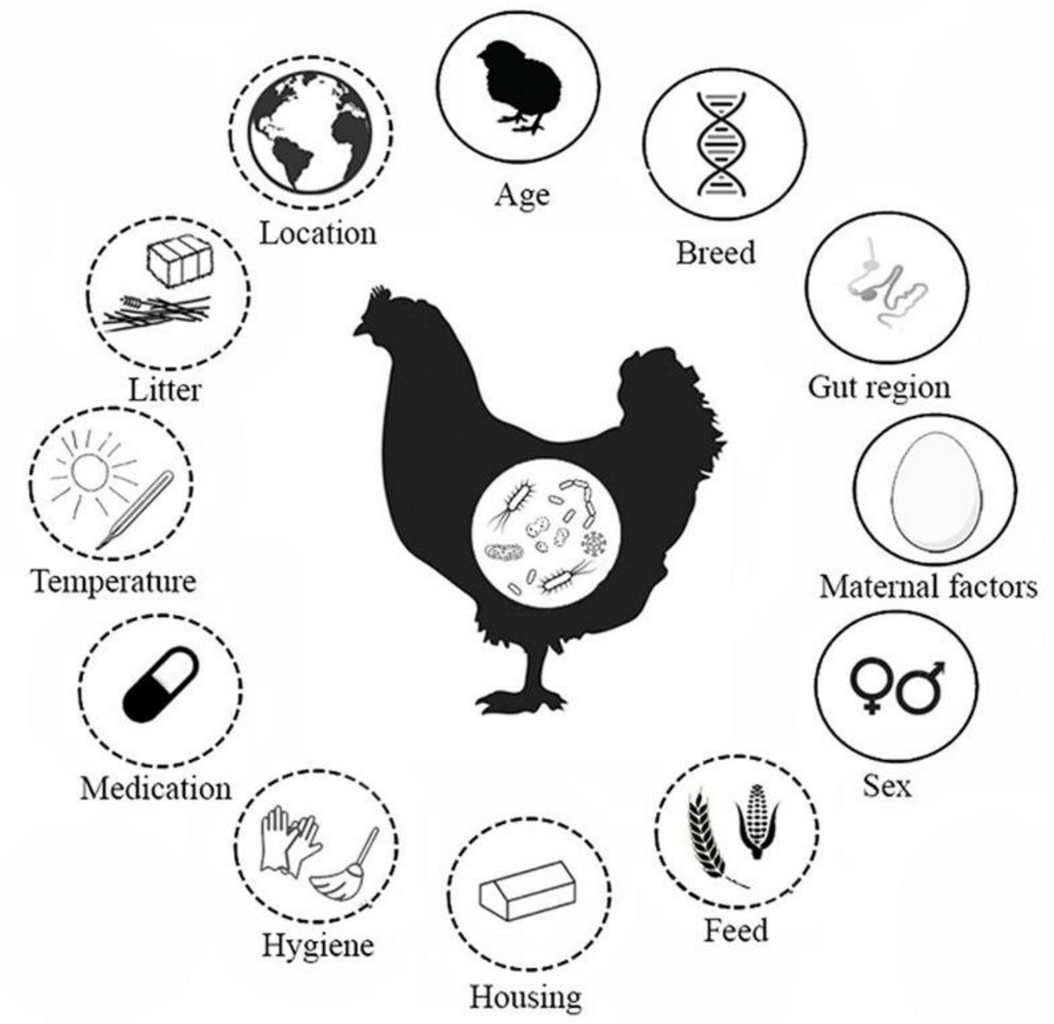
Valeria A. Torok,[1*] Kathy Ophel-Keller,[1] Maylene Loo,[2] and Robert J. Hughes[3]



Effect of microbiome on health

- Resistance/ susceptibility to pathogens
- High / low body weight
- Good/ poor food conversion efficiency
- Nutrient content of food

Factors that affect the microbiome



Chicken Caecal Microbiome Modifications Induced by *Campylobacter jejuni* Colonization and by a Non-Antibiotic Feed Additive

Alexandre Thibodeau, Philippe Fravalo, Étienne Yergeau, Julie Arsenault, Ludovic Lahaye, Ann Letellier

Impact of industrial production system parameters on chicken microbiomes: mechanisms to improve performance and reduce *Campylobacter*

Aaron McKenna, Umer Zeeshan Ijaz, Carmel Kelly, Mark Linton, William T. Sloan, Brian D. Green, Ursula Lavery, Nick Dorrell, Brendan W. Wren, Anne Richmond, Nicolae Corcionivoschi & Ozan Gundogdu

Microbiome 8, Article number: 128 (2020) | Cite this article

RESEARCH Open Access

The chicken gut metagenome and the modulatory effects of plant-derived benzylisoquinoline alkaloids

Peng Huang[1,2†], Yan Zhang[3†], Kangpeng Xiao[1,2†], Fan Jiang[3†], Hengchao Wang[3], Dazhi Tang[4], Dan Liu[4], Bo Liu[3], Yisong Liu[1,5], Xi He[6], Hua Liu[7], Xiubin Liu[1], Zhixing Qing[1], Conghui Liu[3], Jialu Huang[1,5], Yuwei Ren[3], Long Yun[6], Lijuan Yin[3], Qian Lin[6], Cheng Zeng[1,5], Xiaogang Su[5], Jingyang Yuan[5], Li Lin[1,5], Nanxi Hu[1,5], Hualiang Cao[1,2], Sanwen Huang[3], Yuming Guo[4*], Wei Fan[3*] and Jianguo Zeng[1,7*]

Host and Environmental Factors Affecting the Intestinal Microbiota in Chickens

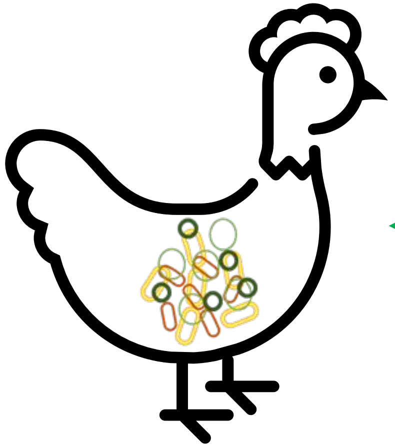
Jannigje G. Kers[1,2], Francisca C. Velkers[1], Egil A. J. Fischer[1], Gerben D. A. Hermes[2], J. A. Stegeman[1] and Hauke Smidt[2]*

[1] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands, [2] Laboratory of Microbiology, Wageningen University & Research, Wageningen, Netherlands

The microbiome as a modifiable factor to improve health

Factors affecting microbiome

- Food/ Diet
- Supplementation
- Antibiotics
- Enclosure/ housing
- Breed
- Weight
- Age
- Presence of other animals in vicinity
- Location
- Bird density



Effect of microbiome on health

- **Resistance**/ susceptibility to pathogens
- **High** / low body weight
- **Good**/ poor food conversion efficiency
- Nutrient content of food

If we know what effects we desire: e.g. pathogen resistance- can we modify farming methods and treatment?

Extremely multivariate data: Challenges

Factors affecting microbiome

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- Supplementation
- Antibiotics
- Enclosure/ housing
- Breed
- Weight
- Age
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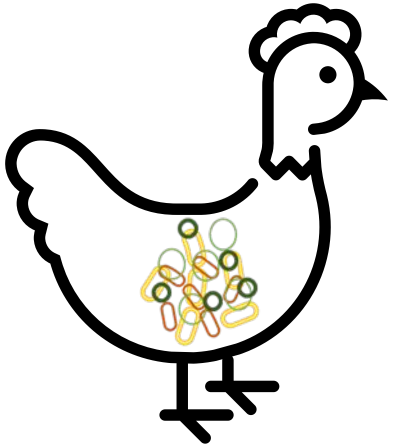

Challenge

1) Individually test the effect of each factor, we lose ability to generalise results

2) If we begin to test permutations of the different factors we quickly lose statistical power

RVC

Treating the microbiome as the variable of comparison

Factors affecting microbiome

- Food/ Diet
- Supplementation
- Antibiotics
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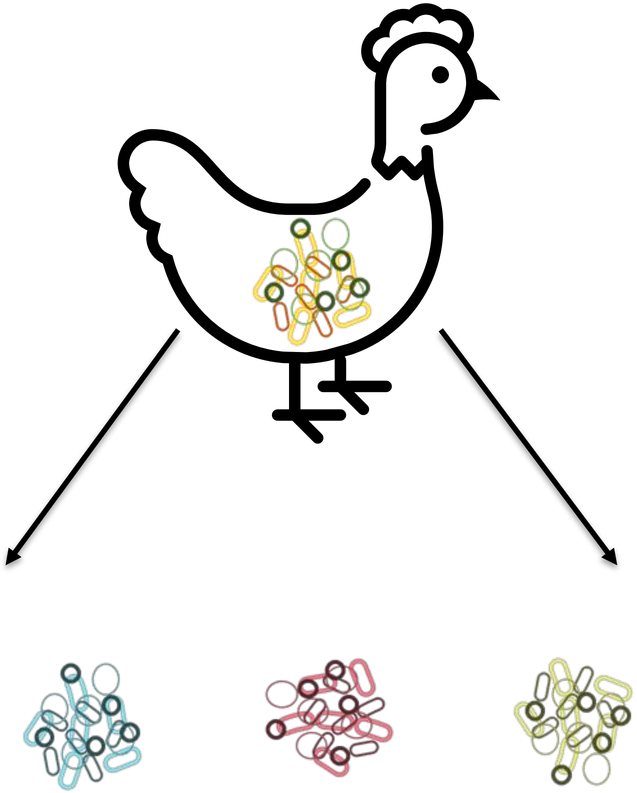

Effect of microbiome on health

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Treating the microbiome as the variable of comparison

Factors affecting microbiome

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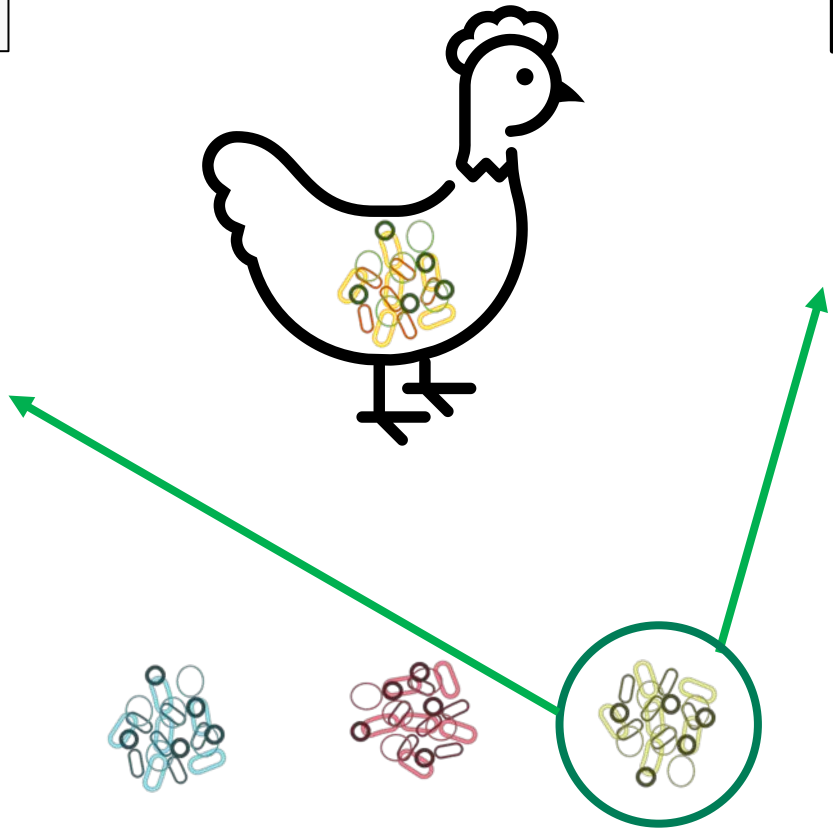

Effect of microbiome on health

- **Resistance**/ susceptibility to pathogens
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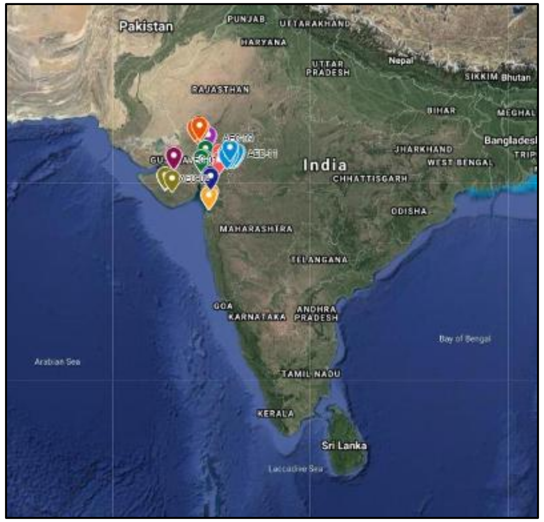
- **Good**/ poor food conversion efficiency
- Nutrient content of food

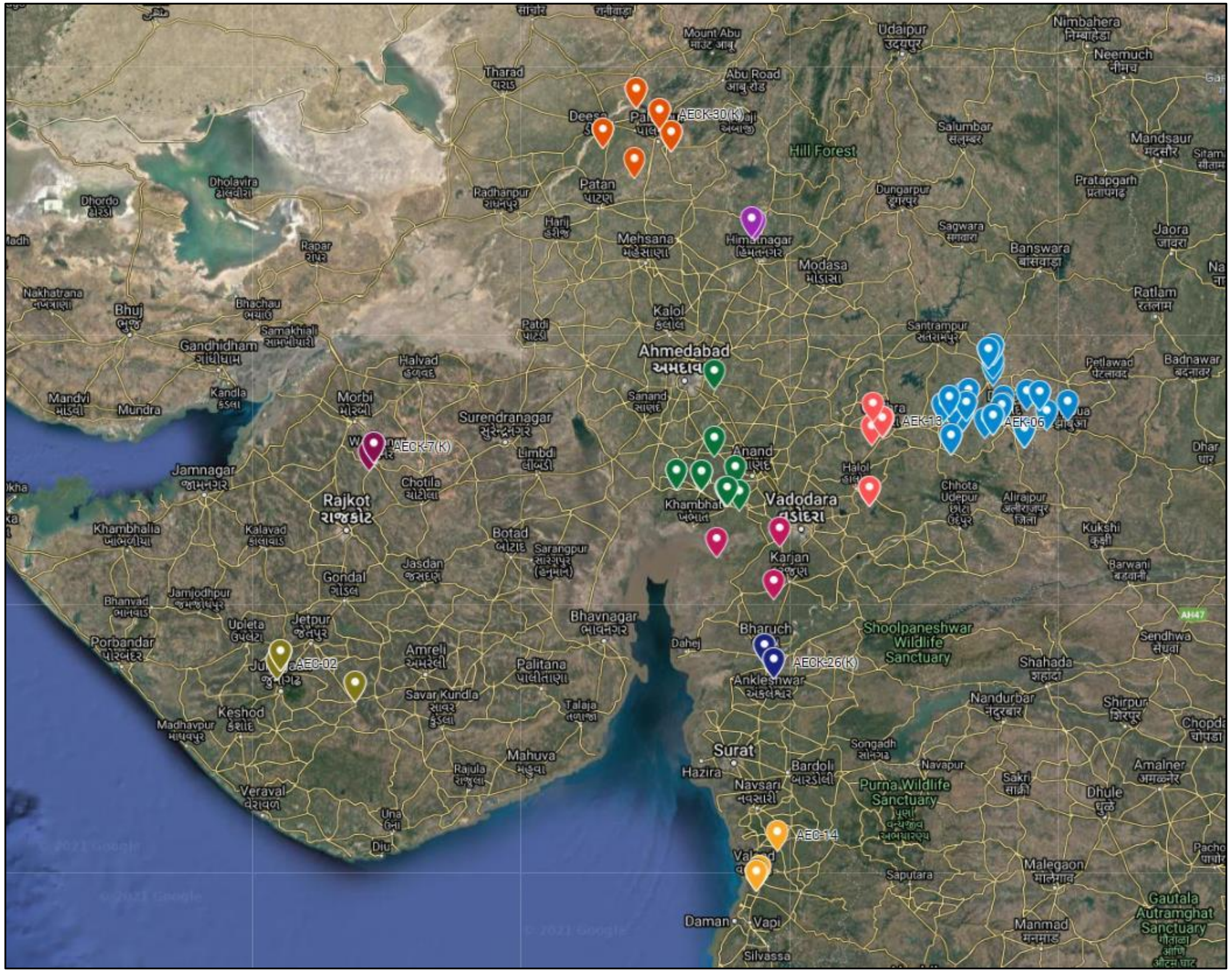
Case Study: Identifying enterotypes in 600 chickens from Northwest India

- Can we identify chicken caecal enterotypes?
- Do these enterotypes have different levels of pathogen abundance?
- Can we describe the community dynamics that contribute to enterotype resilience?
- Do environmental factors contribute to enterotype (and how)?

Case Study: The sites (60 farms)



North West India



Case Study: The Chicken Breeds

Cobb400

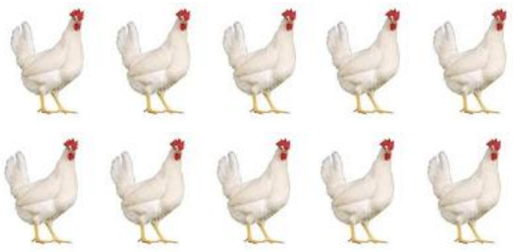
- Commercial broiler line
- Widely used in India
- Descended from Cobb500 and Cobb100 hybrids
- Cobb100 had previously been acclimatized within India

Kadaknath

- indigenous Indian chicken,
- Prized for its “black” meat, Reported to be resistant to some infectious diseases
- Immune parameters distinct from those of modern commercial broiler chickens

Study Design

Cobb only

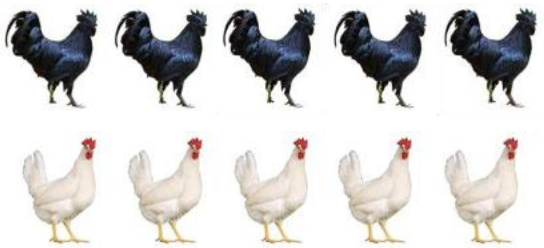


15 farms
150 chickens

Three farm types
10 chickens from each farm

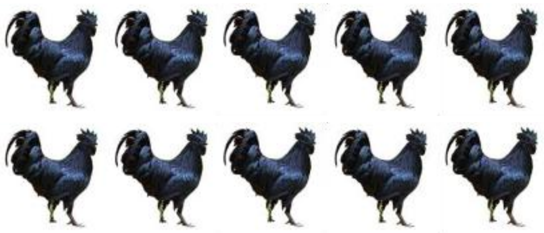
60 farms
600 chickens

Kadaknath + Cobb



30 farms
300 chickens

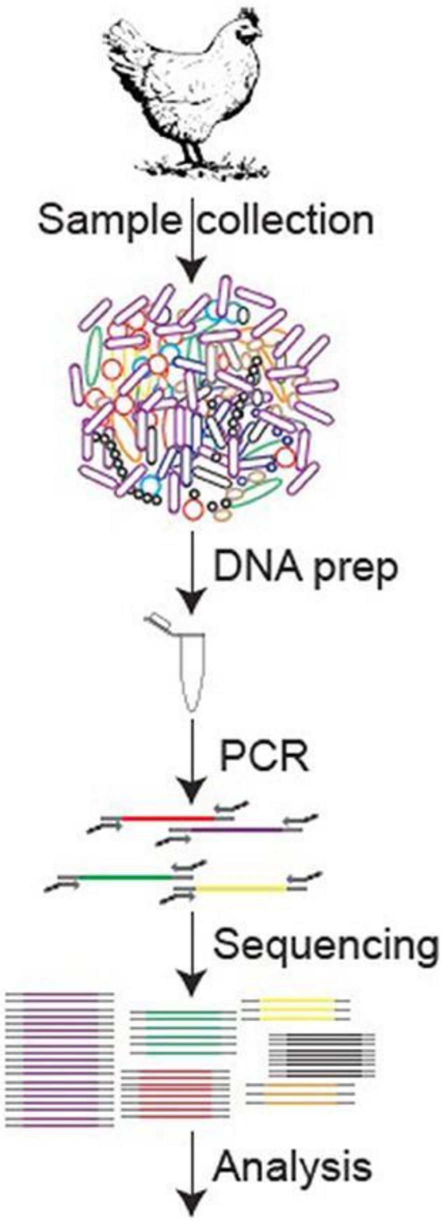
Kadaknath only



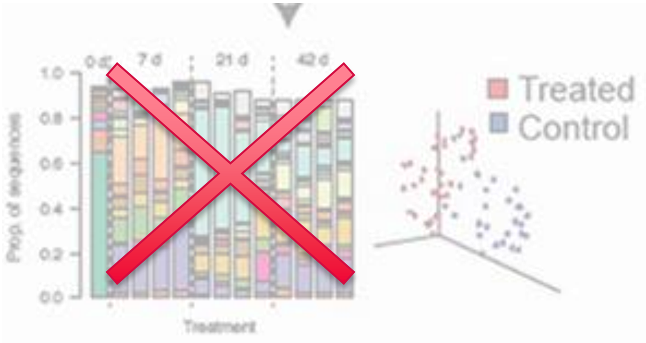
15 farms
150 chickens

+metadata from Questionnaires

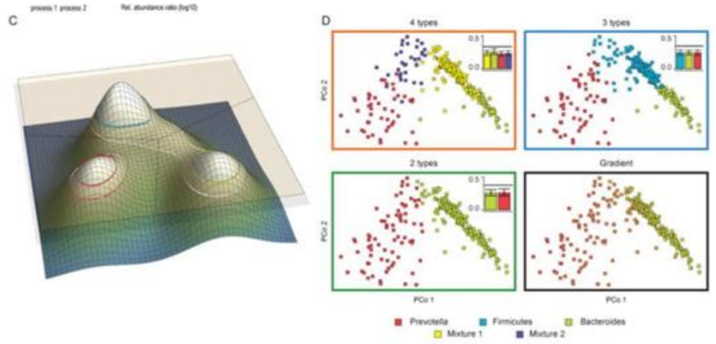
Methods:



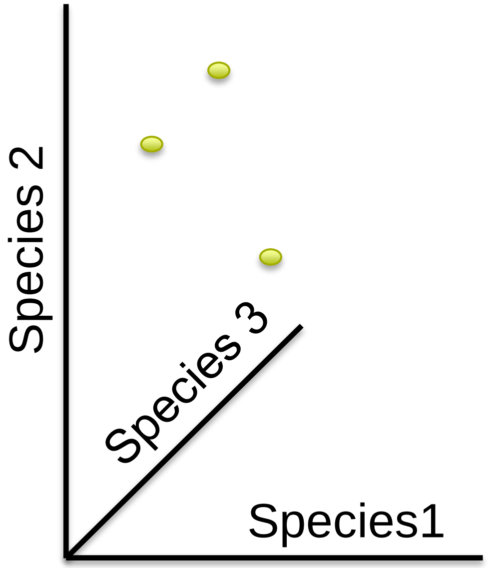
Compare groups by treatment variable, Breed, diet etc.

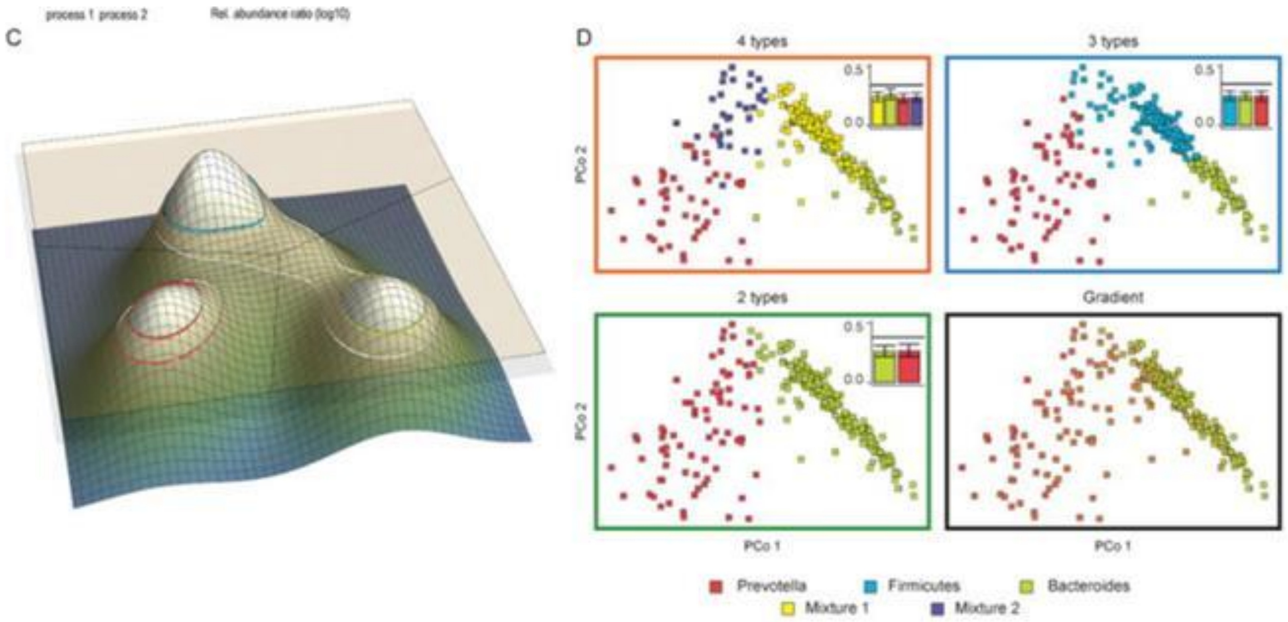


Cluster the sample microbiomes into “enterotypes”. Compare enterotypes.



Enterotypes: decreasing the hugely multivariate data into a manageable microbiome ‘phenotypes’





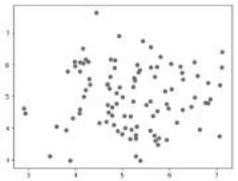
Enterotypes: “densely populated areas in a multidimensional space of community composition” ”

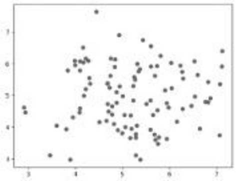
Methods

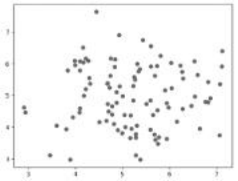
Count data

	Species 1	Species 2	Species 3	Species 4	Species 5	Species 6	Species 7	Species 8	...	Species 30
Chicken 1	12056	446	19	10009	348975	4578	333	4566		4566
Chicken 2	127	1244	5681	3456	11	885	4599	4423		4423
Chicken 3	12	222	7568	1178	118	1234	165	371		371
Chicken 4	578	1289	0	1550	222	1308	1111	904		904
Chicken 5	3447	34	63	7322	2512	296	67	1223		1223
Chicken 6	1569	246	465	2301	738	3	12	2		2
Chicken 7	227	8576	1985	44	55	99	34	67		67
Chicken 8	221	938	8726	2005	77	529	8634	88		88
Chicken 9	9965	688	700	12765	8591	11	12	1155		1155
Chicken 10	88	567	2104	2400	66	86	90	909		909

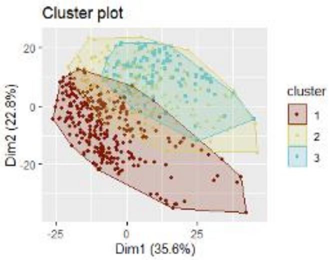
Distance (betadiversity)

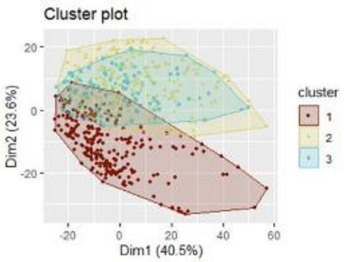


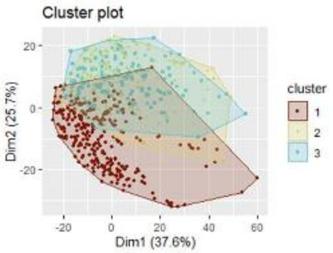




Cluster samples







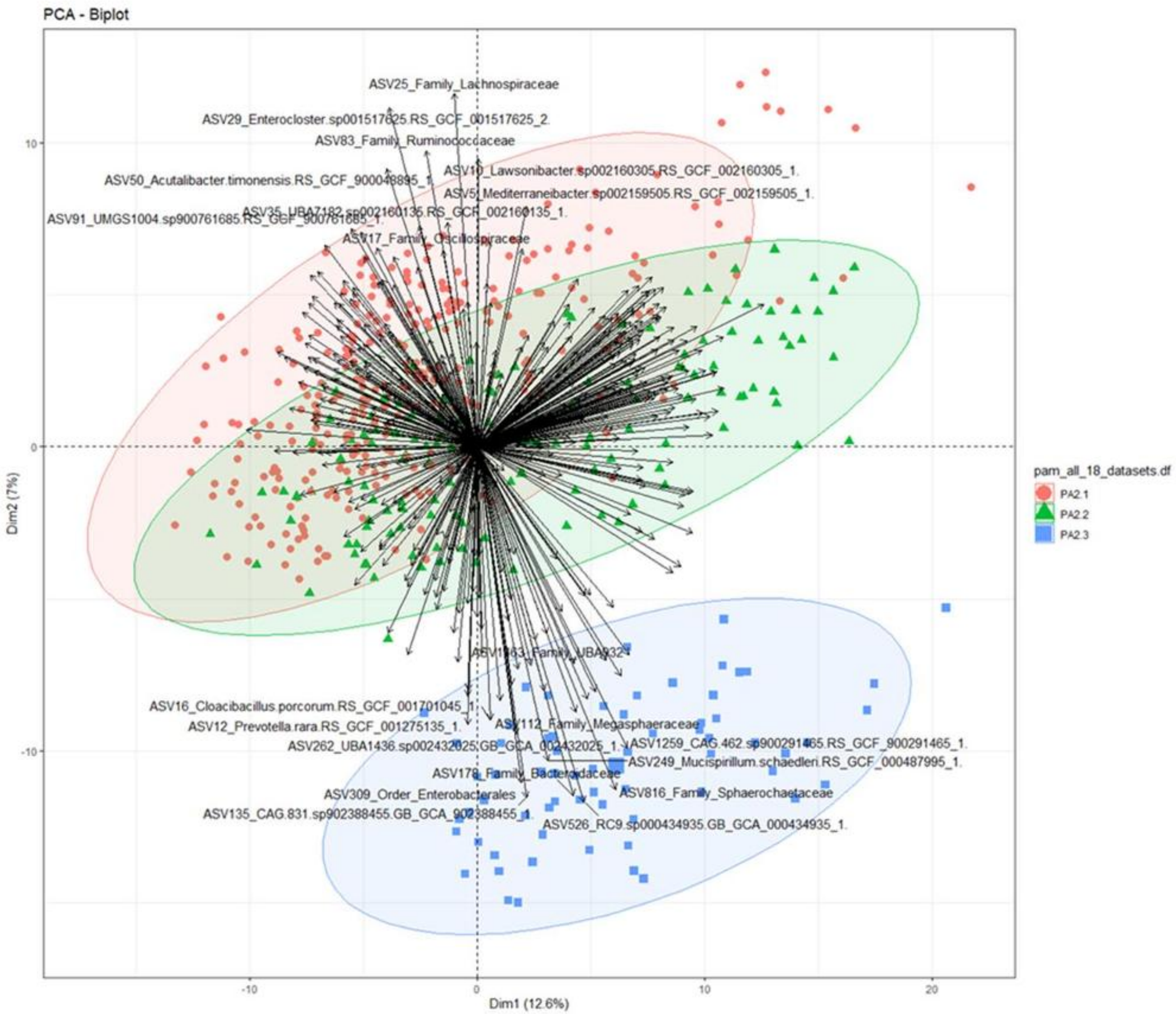
Compare the clusters (Enterotypes)

Enterotype1

Enterotype 2

Enterotype 3

Results: we identified three enterotypes



Three 'enterotypes'

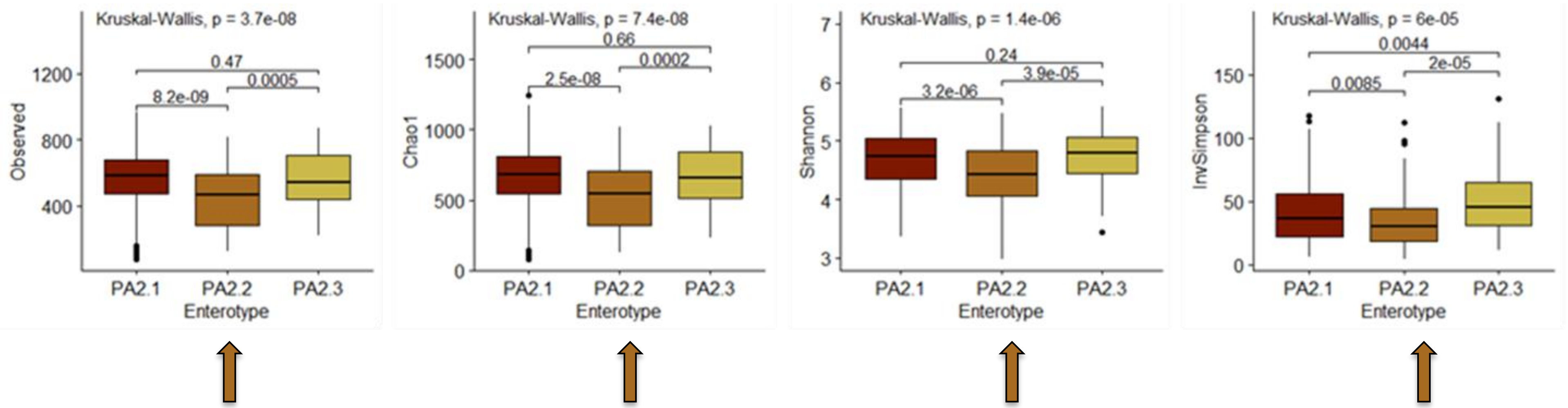
E1: n=290 chickens

E2: n= 142 chickens

E3: n= 67 chickens

RVC

Alpha diversity: how many different types of bacteria are there?



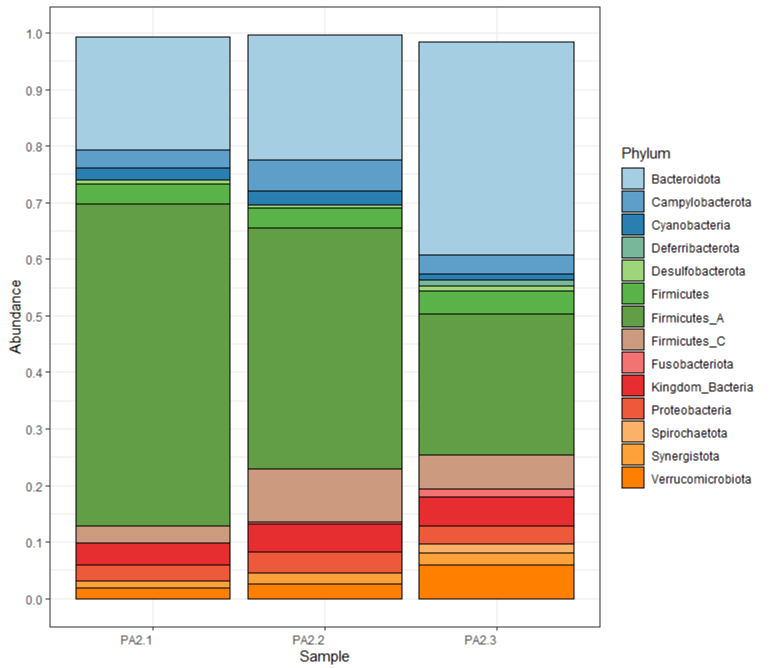
- Alpha diversity measures the diversity within a single sample. (species richness)
- Chickens with Enterotype 2 have lower diversity compared to other groups.

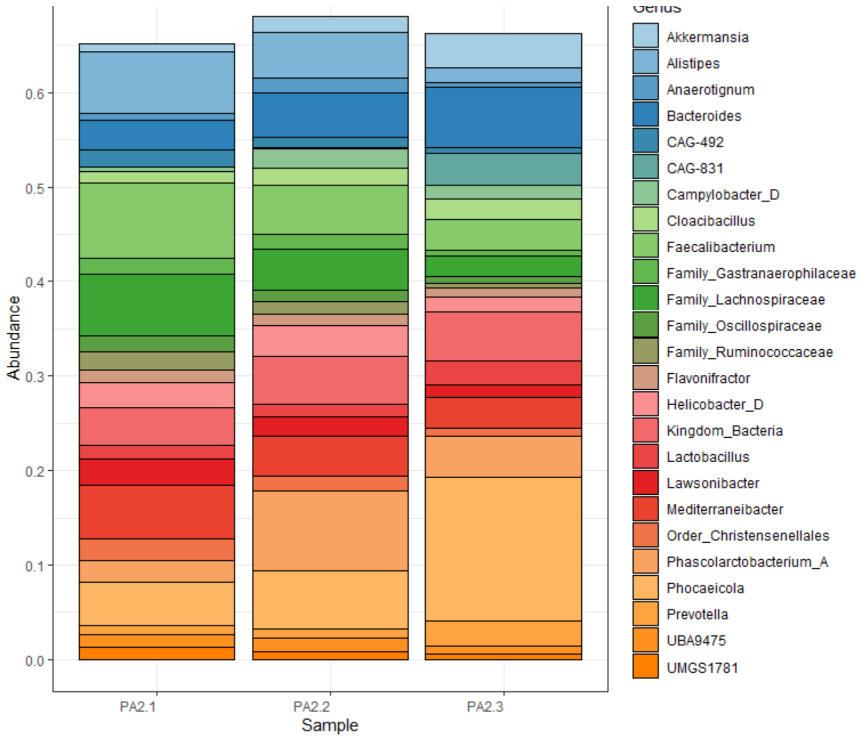
- This is important because high alpha diversity is often linked to resilience, an low alpha diversity is sometimes observed in disease states.

Results: Relative abundance at Phylum and Genus

Firmicutes vs Bacteroides ratio

- E1 has the highest ratio of Firmicutes to Bacteroides
- *Faecalibacterium* highest genus in E1- butyrate producer, associated with health
- Bacteroides in some autoimmune diseases, and maybe increased in birds with Coccidiosis
- *Phocaeicola dorei* high in E2
- CAG-831 / *Alistipes* – E3
- *Campylobacter* highest in E2, E3





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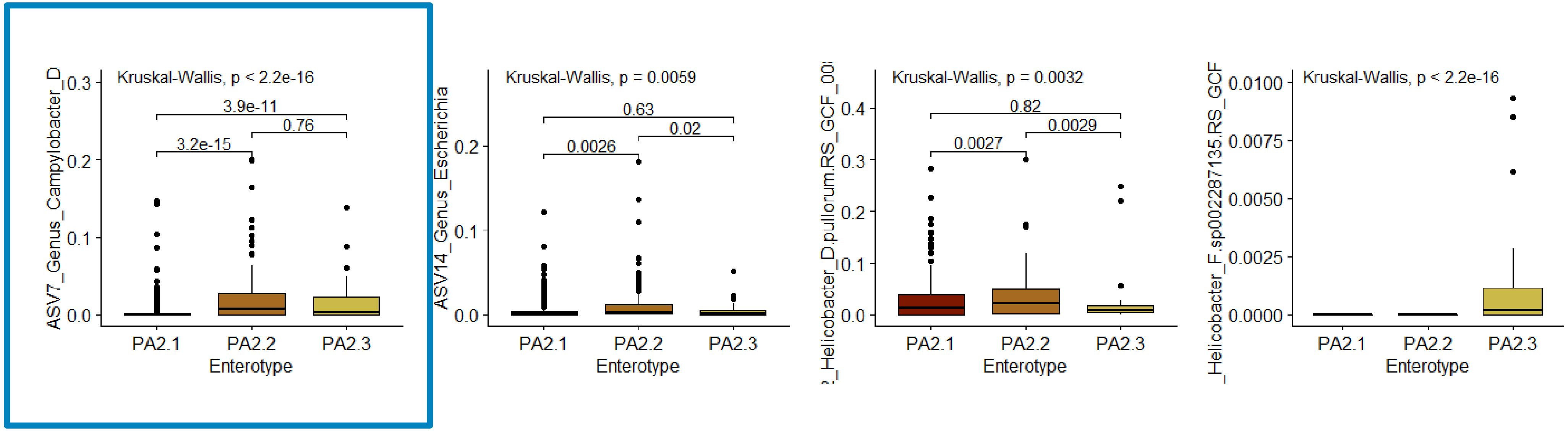
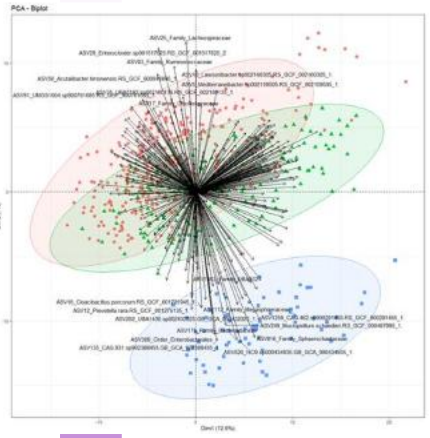
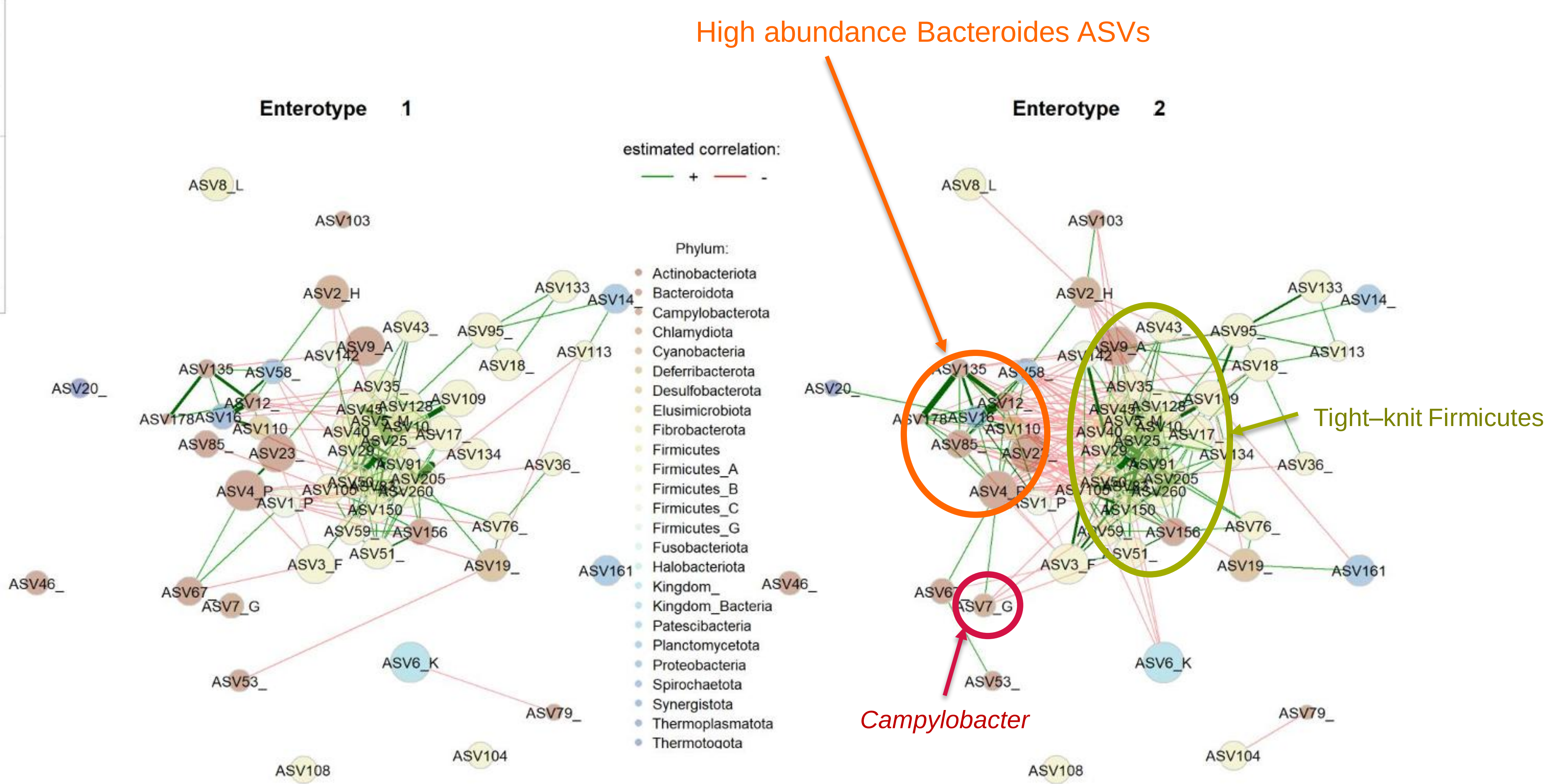
Presence of pathogens



Comparison of relative abundance of potential pathogens in chicken enterotypes. The mean RA of *Campylobacter D* which includes *Campylobacter coli* and *jejuni* significantly higher in E2.

Network analysis to understand community interactions





The Bacteroides are negatively associated with the Firmicutes species that normally inhibit *Campylobacter*

Can we predict enterotype from farming characteristics?

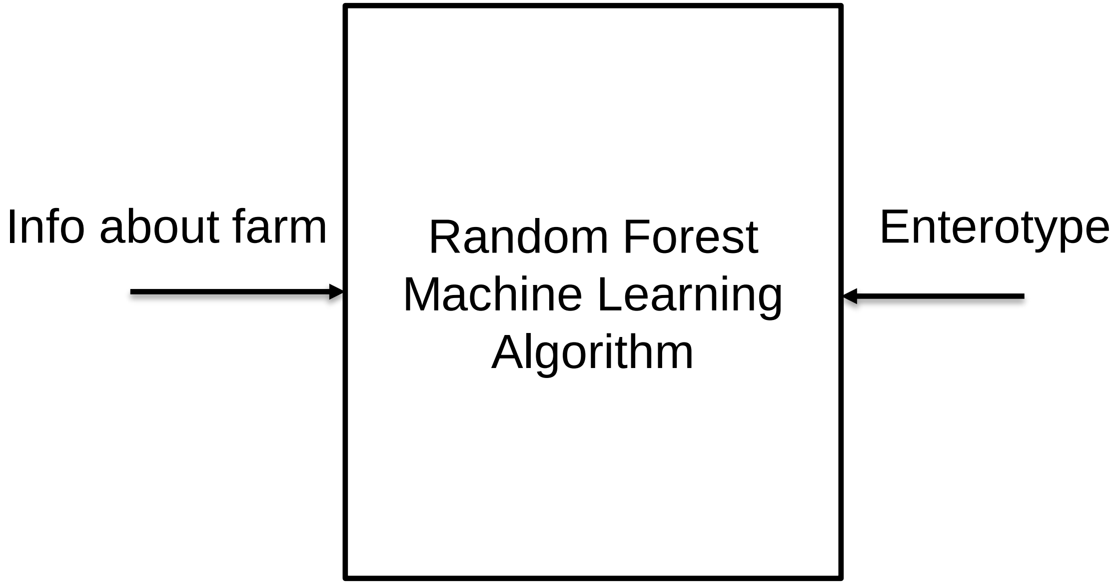
Yes! (Kinda)

Can we predict enterotype from farming characteristics?

Yes! (Kinda)

Machine learning algorithms:
Split data in two: training, and testing.

Train



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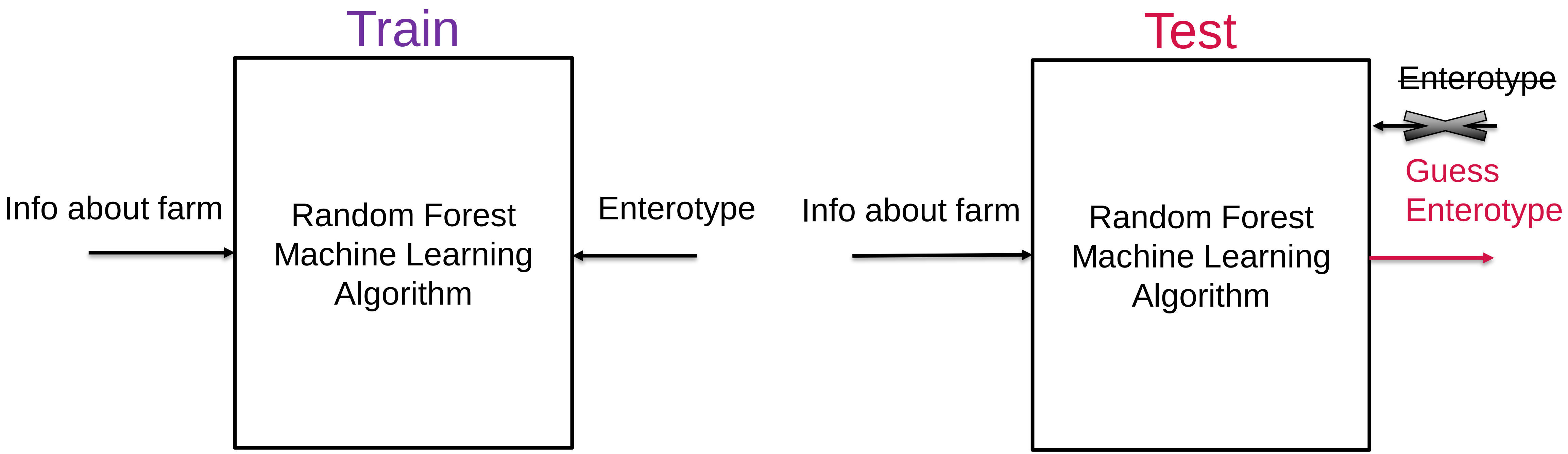

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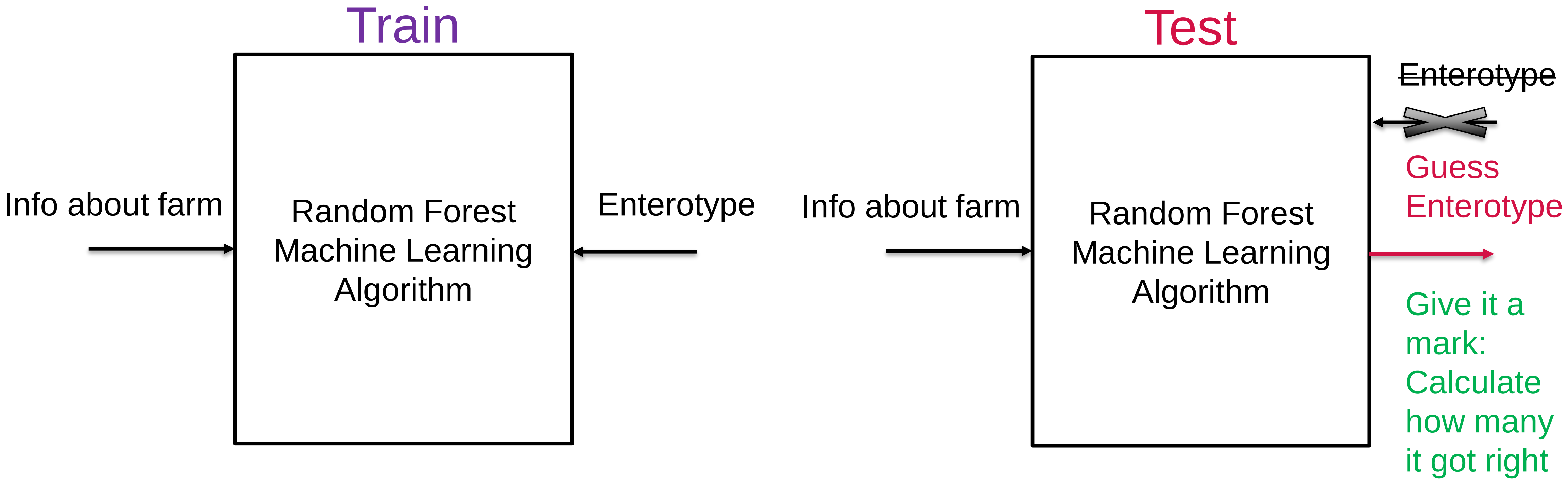

Our Random Forest Assessment

52-58% accurate

Not great, but significantly better than a random guess.

Why isn't it better?

- Data might not be reliable
- We didn't feed it enough data

- Mainly- there are MANY factors we haven't accounted for

Model 1

Confusion Matrix and Statistics

Prediction \ Reference	PA2.1	PA2.2	PA2.3
PA2.1	**61**	20	31
PA2.2	7	**24**	9
PA2.3	2	8	**0**

Model 2

Confusion Matrix and Statistics

Prediction \ Reference	PA2.1	PA2.2	PA2.3
PA2.1	**65**	23	37
PA2.2	5	**27**	1
PA2.3	0	2	**2**

	Model 1			**Model 2**		
Overall Statistics						
Accuracy	0.5247			0.5802		
95% CI	(0.4449, 0.6036)			(0.5003, 0.6572)		
No Information Rate (NIR)	0.4321			0.4321		
P-Value [Acc > NIR]	0.01102			0.000105		
Kappa	0.2167			0.2947		
Mcnemar's Test P-value	5.76E-07			1.37E-10		
Statistics by Class						
	Class: PA2.1	Class: PA2.2	Class: PA2.3	Class: PA2.1	Class: PA2.2	Class: PA2.3
Sensitivity	0.8714	0.4615	0	0.9286	0.5192	0.05
Specificity	0.4457	0.8545	0.91803	0.3478	0.9455	0.98361
Pos Pred Value	0.5446	0.6	0	0.52	0.8182	0.5
Neg Pred Value	0.82	0.7705	0.73684	0.8649	0.8062	0.75949
Prevalence	0.4321	0.321	0.24691	0.4321	0.321	0.24691
Detection Rate	0.3765	0.1481	0	0.4012	0.1667	0.01235
Detection Prevalence	0.6914	0.2469	0.06173	0.7716	0.2037	0.02469
Balanced Accuracy	0.6585	0.658	0.45902	0.6382	0.7323	0.5168

Factors affecting enterotype

E1 :Farms that DON'T allow visitors inside, don't have other birds on the farm.

E2: Farms with dogs, that allow visitors inside, and have other birds.

E3: Farms that have other birds on the farm, store their feed outside.

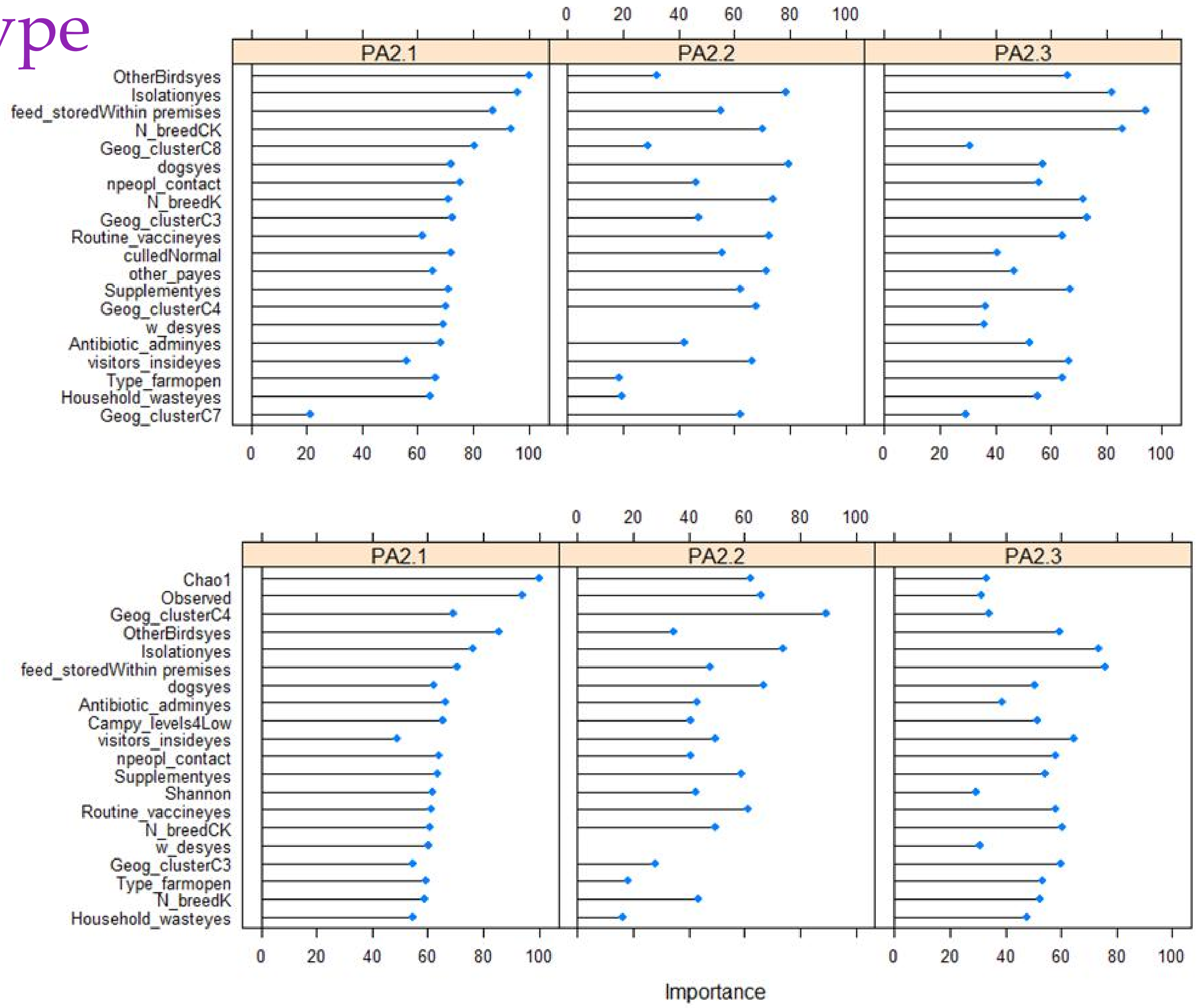

Figure 15(top) Variable importance for each enterotype, using a RF model constructed from farming practice variables (21) geographical region only. (bottom) Variable importance for enterotype using a RF model constructed from farming practice and geographical region and alpha diversity measurements.

What we can conclude

- This a promising approach

- Identified three enterotypes
  - E1: largest group, high species richness, high Firmicutes relative to Bacteroides, low *Campylobacter*, high *Faecalibacterium* – *a resilient enterotype?*
  - E2: low species richness, high *Campylobacter,* higher Bacteroides relative to Firmicutes – *a susceptible enterotype?*
  - E3: high alpha species richness, high *Campylobacter*, higher Bacteroides relative to Firmicutes, several unique genera. Hard to know!

RVC

Why this is cool

- *Campylobacter* prevalence is VERY difficult to control.
- *Campylobacter* **abundance** may depend on enterotype.
- This work shows that enterotype might affect *Campylobacter* abundance AND elucidates some of the community dynamics that may be at play.

RVC

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Factors affecting microbiome

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- Supplementation
- Antibiotics
- Enclosure/ housing
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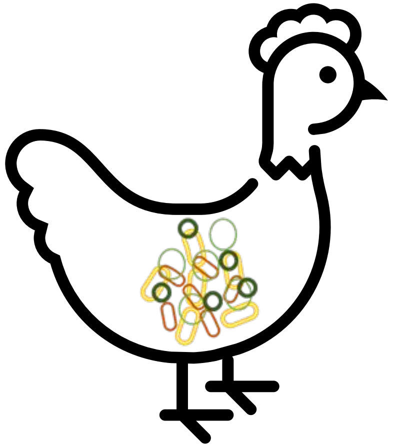
- Age
- Presence of other animals in vicinity
- Location
- Bird density



Effect of microbiome on health

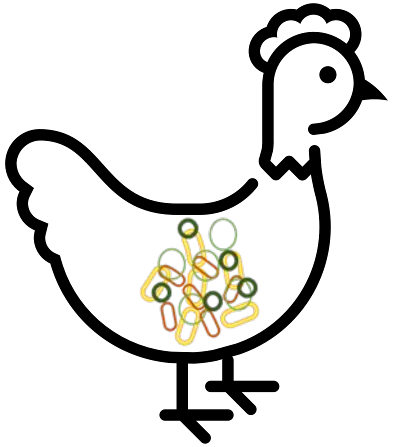
- **Resistance**/ susceptibility to pathogens
- **High** / low body weight
- **Good**/ poor food conversion efficiency
- Nutrient content of food

Do it again, on a different dataset, with more samples and more variables

Factors affecting microbiome

- Food/ Diet
- Supplementation
- Antibiotics
- Enclosure/ housing
- Breed
- Weight
- Age
- Presence of other animals in vicinity
- Location/ Geography
- Bird density

+ more breeds
+ more countries
+ detailed antibiotic info
+ detailed feed info



Effect of microbiome on health

- **Resistance**/ susceptibility to pathogens
- **High** / low body weight
- **Good**/ poor food conversion efficiency
- Nutrient content of food

+ examples of sick birds
+ phenotype data (weight, condition etc)

ONE HEALTH POULTRY HUB

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