


Microbial community analysis of the ruminant gut and potential impact on pathogen colonisation and excretion.

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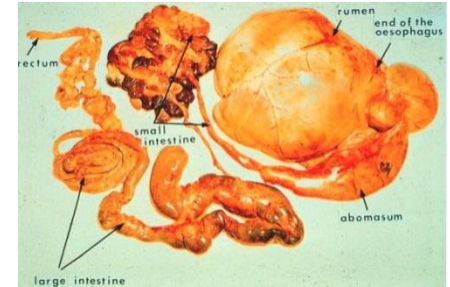
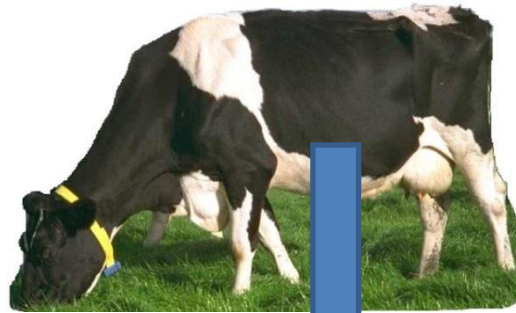
IDReC Symposium, Victoria University, 9-10 September 2014.



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Presentation outline

- Microbial community analysis of the ruminant and human gut – culture-independent metagenomic studies
- Association of pathogen and commensal microbial taxa
- Cross-sectional and longitudinal culture (-independent) studies



Bacteria

~400 species
 10^{10} to 10^{11} cells/ml



Anaerobic Fungi

~30 species
 $<10^5$ cells/ml



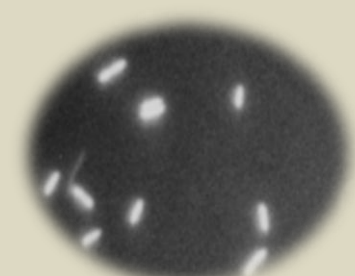
Ciliate Protozoa

~40 species
 $<10^5$ cells/ml



Methanogenic Archaea

~6 species
 10^6 to 10^8 cells/ml



- Essential for ruminants to effectively utilise dietary material through SCFA production.
- Fermentation of feed in the rumen: 60 – 80% of animal's energy requirements.
- Large intestine: 10-20% energy requirements.

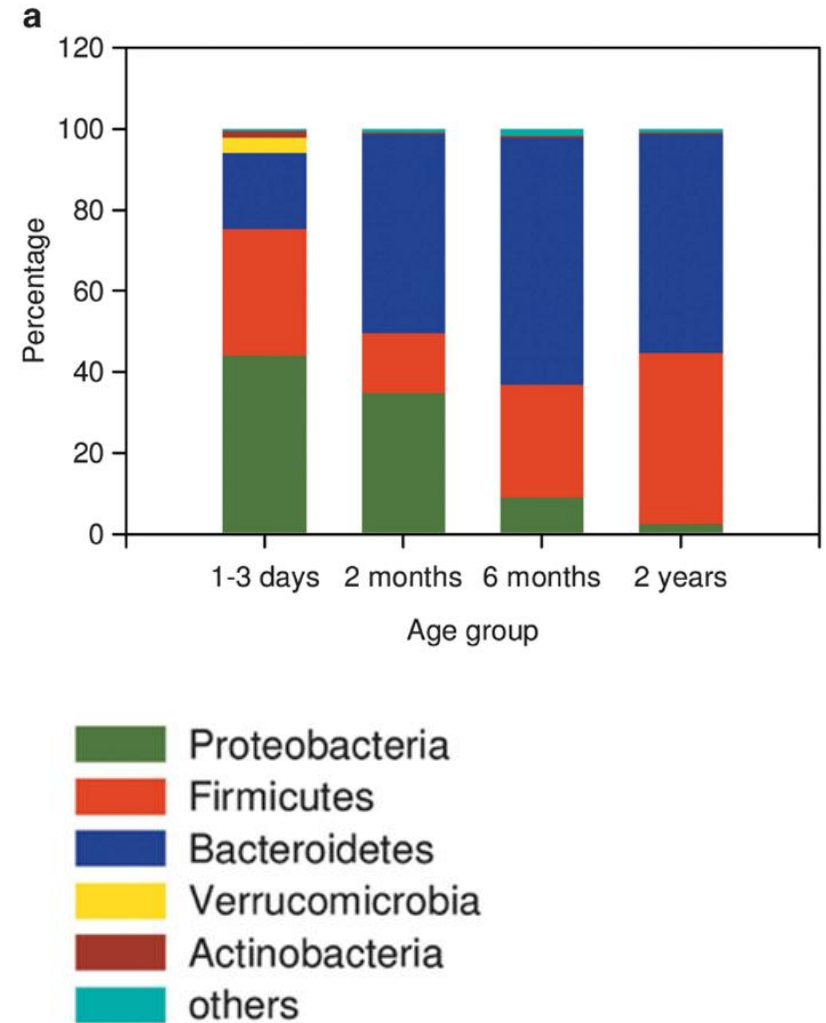
Factors influencing the ruminant microbiome

- Animal age
- Feed type
 - Soluble/insoluble carbohydrate, protein
 - Intake patterns
- Host genetics/gut physiology
- Health/immune status



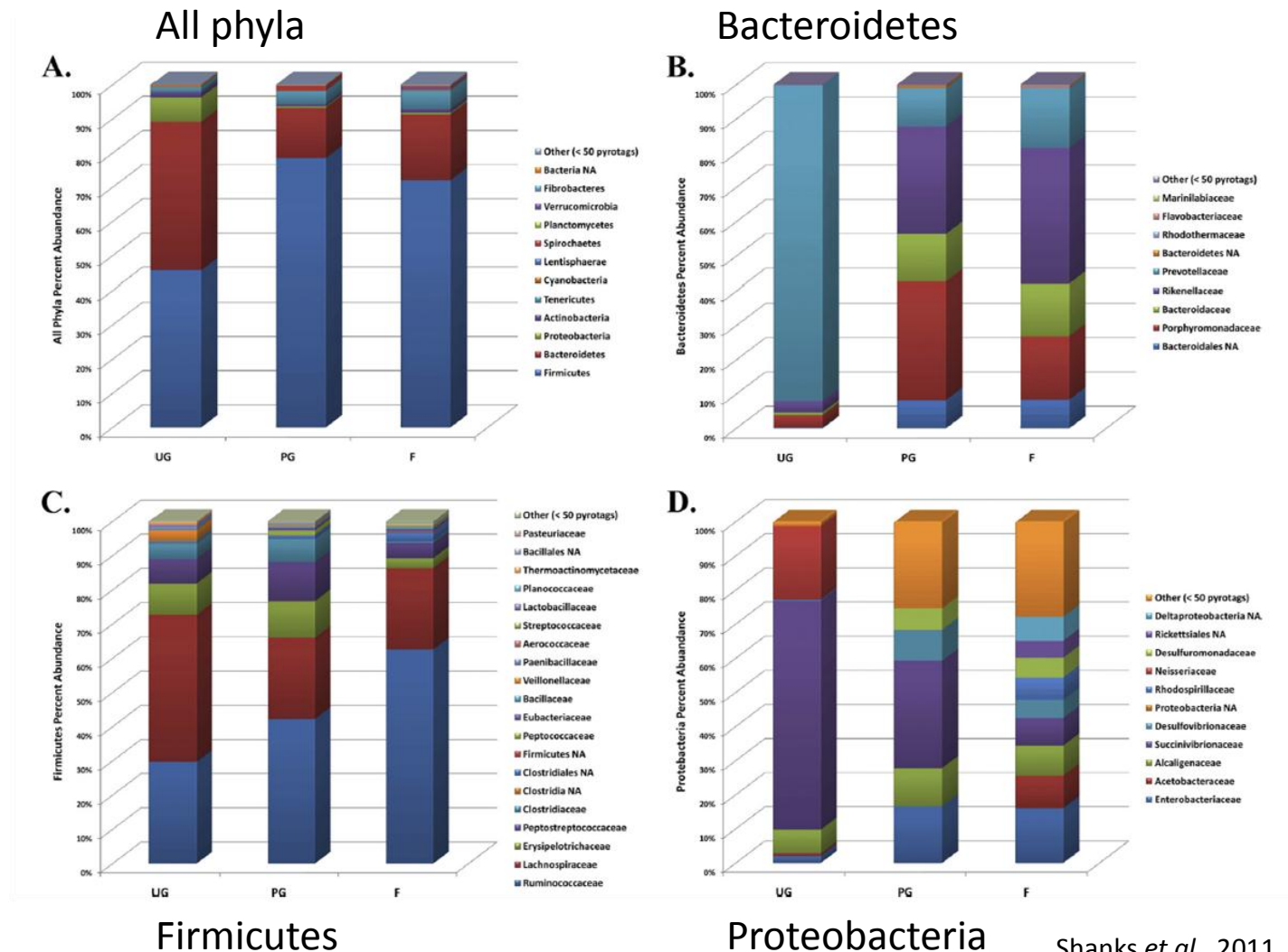
Age

- Rumen microbiota in weaned animal dominated by Firmicutes and Bacteroidetes phyla
- Specialised in function for feed degradation



Jami *et al.*, 2013. ISME, 7:1069-79.

Feed – bovine faecal samples



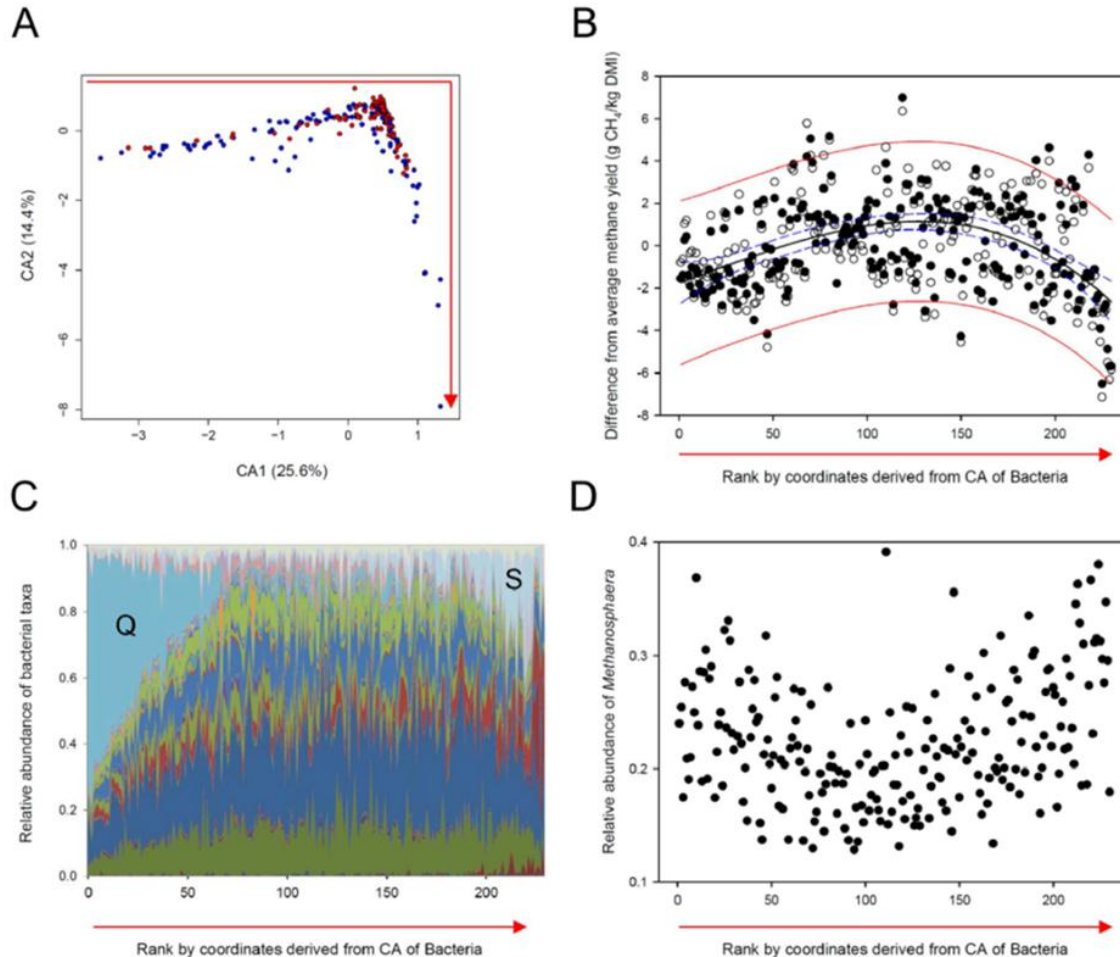
Shanks *et al.*, 2011. AEM, 77:2992-3001.

Host genetics/gut physiology

- Enteric methane production 35% NZ GHG emissions
- Ruminant methane emissions phenotype is heritable
- Selection lines for high and low methane emitting sheep have been developed
- Low methane animals have smaller rumen and shorter feed retention time

Groups	High ME (n=10)	Low ME n=10	Pooled SEM	P
Rumen content weight (kg)	5.42	4.43	0.257	0.074
Rumen volume (litres)	7.42	5.91	0.355	0.048

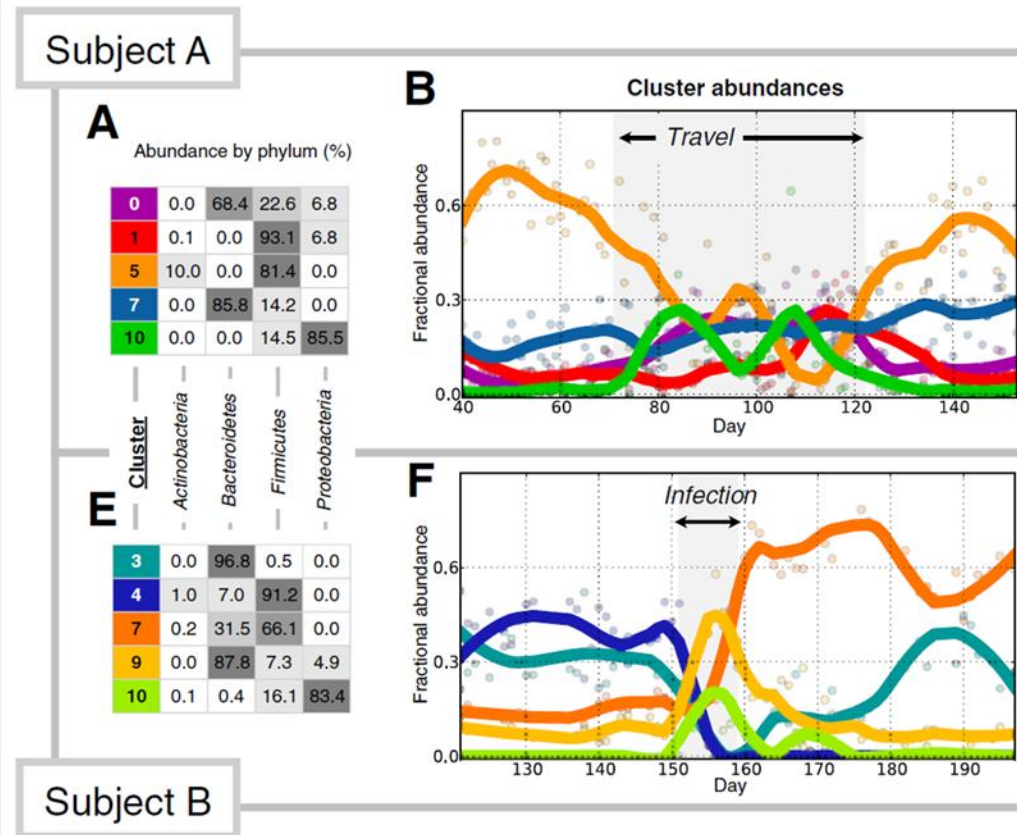
Rumen bacterial community types associated with the contrasting methane emission trait in sheep



Kittelman *et al.*, 2014. PLoS ONE, 9: e103171

Longitudinal studies of the human gut microbiota: lifestyle impacts

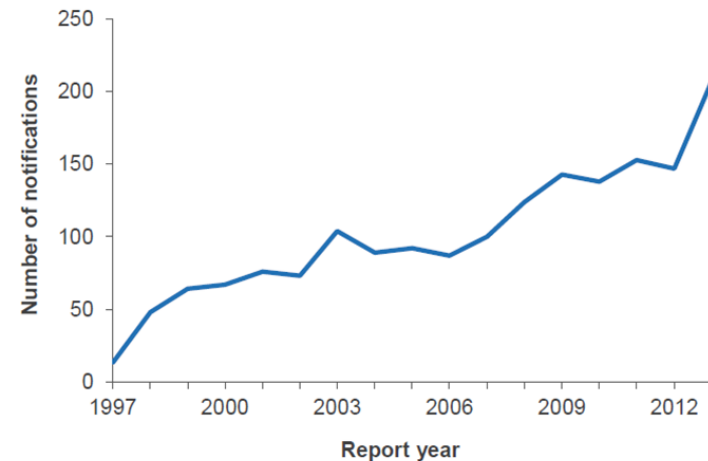
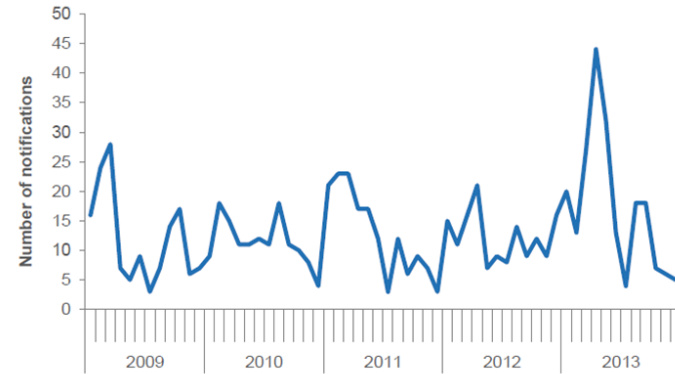
- Faecal samples provided by two healthy males over one year
 - Microbial community analysis
- 10,000 wellness metadata measurements (diet, sleep, exercise, mood)
- Perturbations
 - Subject A – travel/diarrhoea
 - Subject B – Salmonella infection (no antibiotics)





Shiga toxin-producing *Escherichia coli* (STEC) notifications in New Zealand by year, 1997-2013

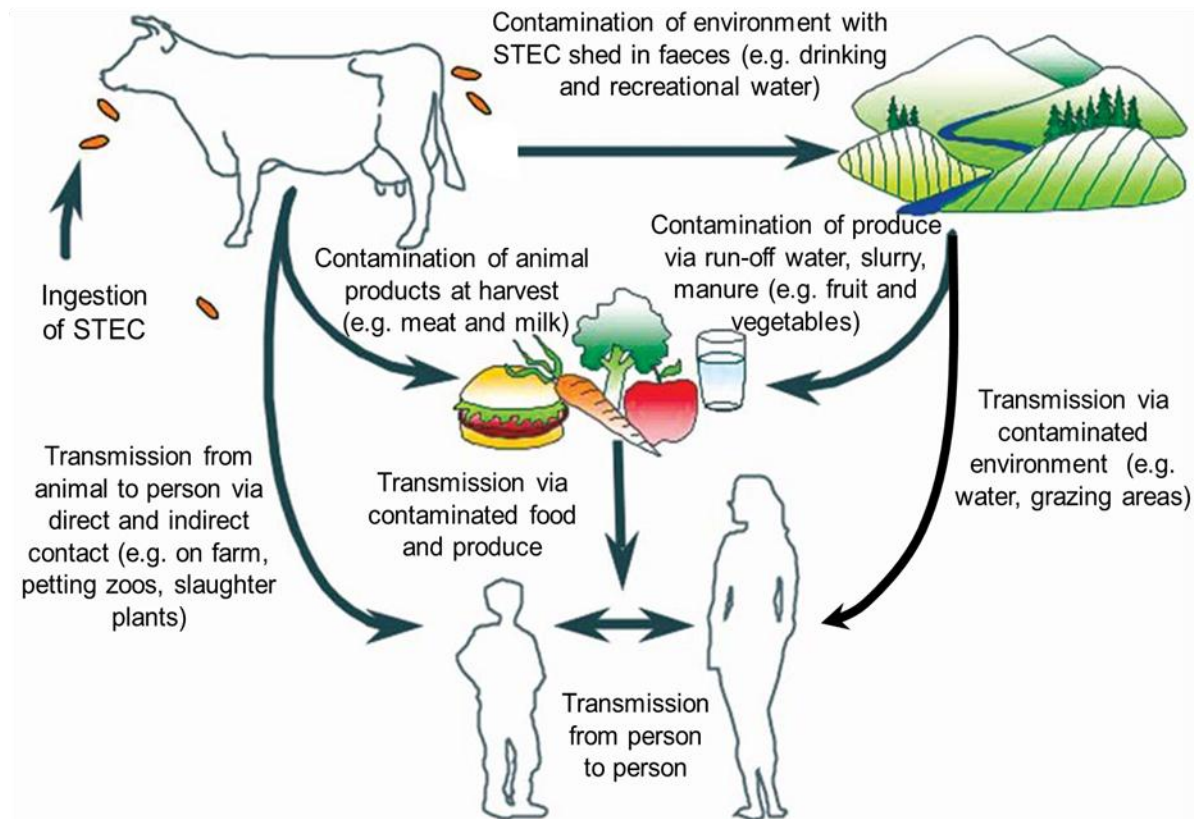
- STEC – zoonotic pathogen
 - Diarrhoea
 - Haemolytic uraemic syndrome
 - Seasonal pattern
- Large foodborne outbreaks of (diarrheal and HUS) disease overseas



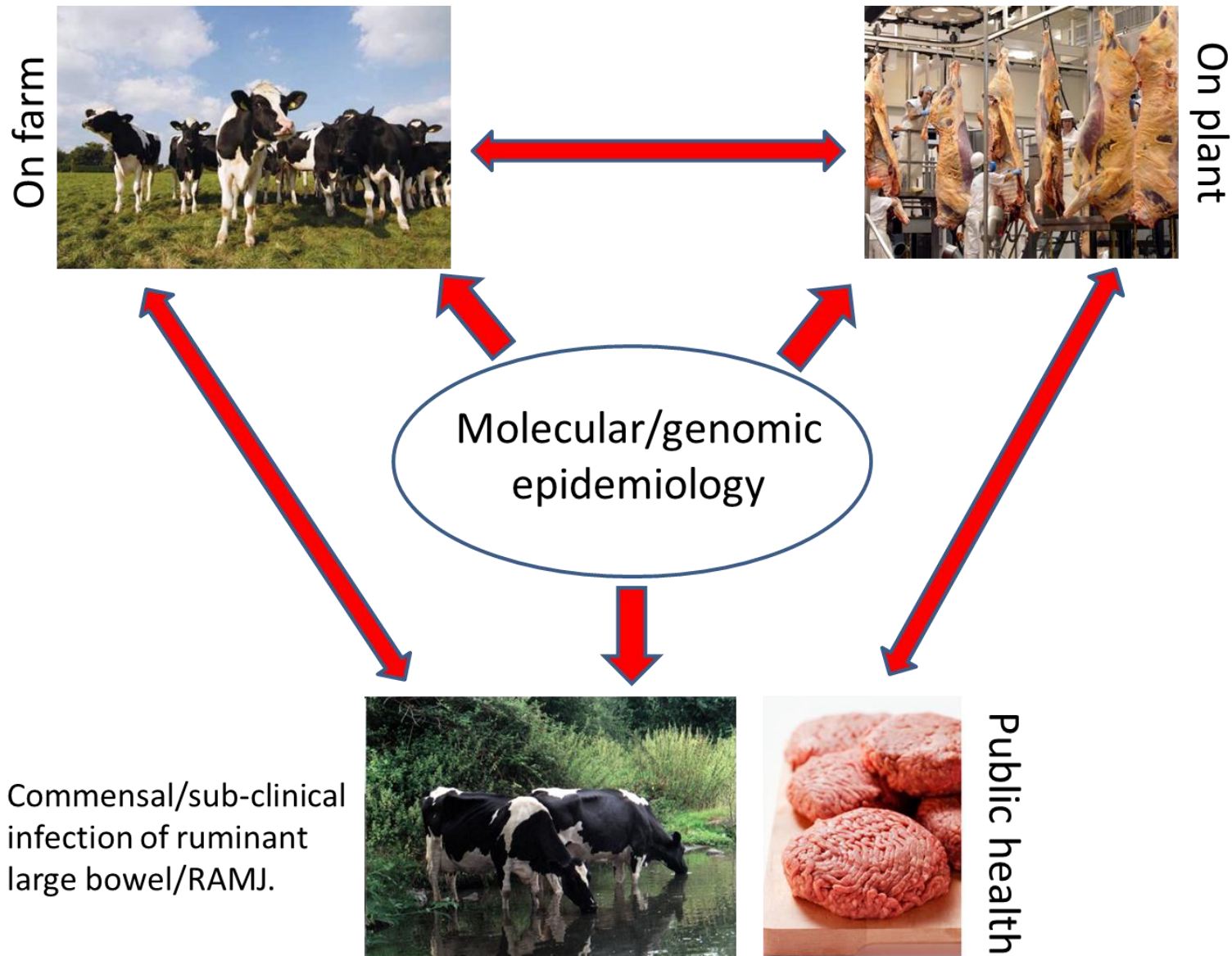
2013. Incidence of 5.1 STEC cases per 100,000 population.
(US: 1.1 per 100,000; Australia: 0.4 per 100,000; Ireland: 6.1 per 100,000; Scotland: 4.5 per 100,000).

STEC contamination and transmission pathways

- O157 serogroup associated with 80-90% STEC clinical cases in NZ
- Isolated cases or sporadic household-associated outbreaks
- Risk factors – contact with animals/animal faeces/environment
- Extensive meat product testing required for continued access to overseas markets



From P. Jaros. Modified from www.ecl-lab.ca



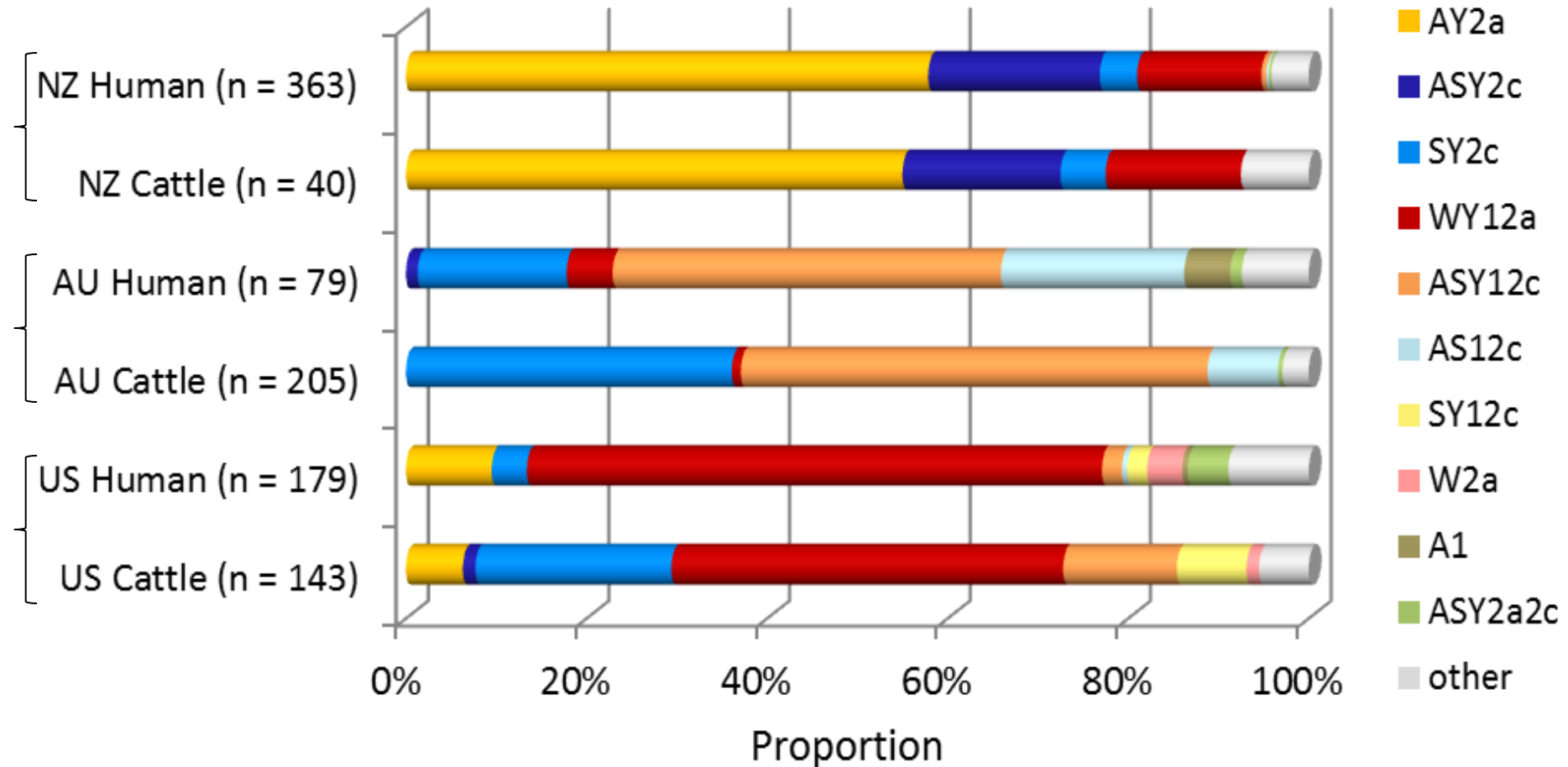
Prevalence of O157 and O26 carriage in NZ slaughter cattle

- Repeated cross-sectional study with sampling at 2 NI and 2 SI NZ slaughter plants (July 2009 – July 2011)
- Collection of recto-anal faecal swabs from carcasses

	Serotype	PCR+ve	Culture+ve	STEC
Adult cattle	O157	7.1% (63/883)	2.5% (22/883)	1.6% (14/883)
	O26	7.7% (68/883)	3.2% (28/883)	0.5% (4/883)
Calves	O157	23.5% (163/695)	3.0% (21/695)	2.3% (16/695)
	O26	33.2% (231/695)	8.6% (60/695)	4.0% (28/695)

- Prevalence of STEC shedding in calves > adult cattle

SBI-typing of STEC O157



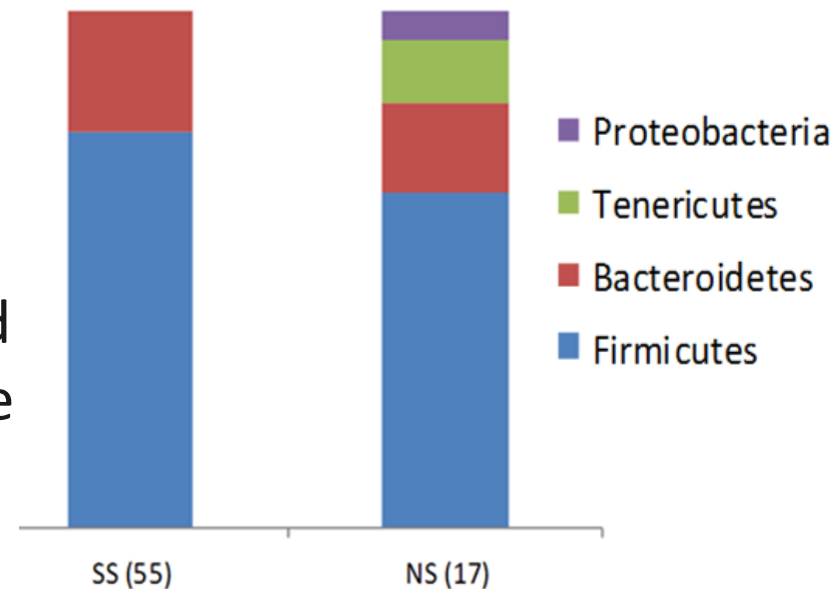
STEC O157 carriage and transmission

- Cross-sectional studies identified cattle described as ‘super-shedders’ – animals excreting $\geq 10^4$ CFU/g faeces
 - <10% of cattle in herd – 90% excretion
 - High risk for contamination/transmission
- Microbial community analysis of single faecal samples taken from 11 SS versus 11 NS cattle from feedlot system



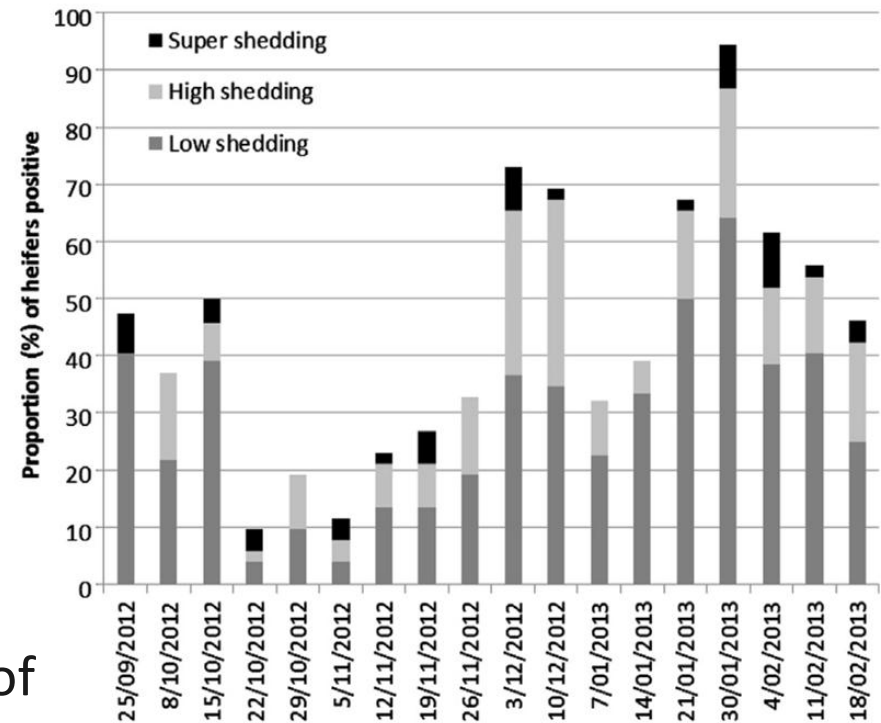
Microbiome from SS and NS cattle

- 112,926 usable 16S rRNA sequences
- 17 OTUs (23.6%) were more abundant in NS
- 55 OTUs (76.4%) were more abundant in SS
- *E. coli*-associated OTUs represented 0.015% of the dataset (17 sequence reads)
- Only 38.6% (97% similarity) of the OTUs could be mapped onto reference OTUs (Greengenes)



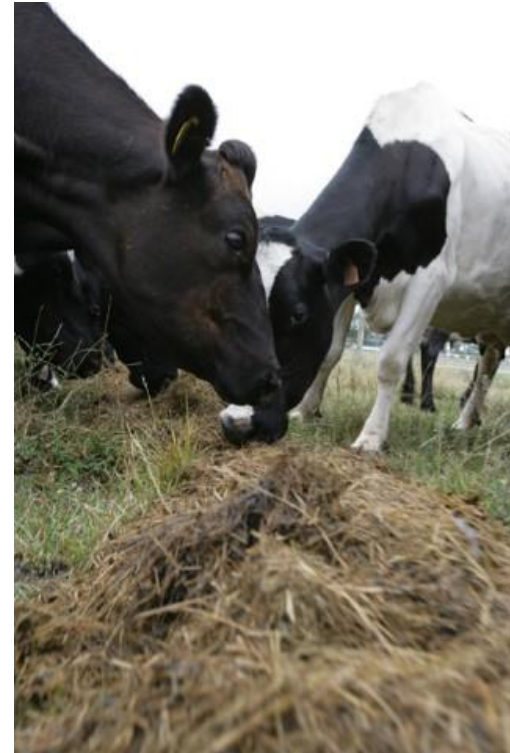
Prevalence and SS of STEC O157 – longitudinal study

- 52 heifers on pasture
- All provided at least one faecal sample positive for STEC O157
- 24 SS at some point over 18 **weekly** sampling points
- 19 animals detected as SS on single occasion
- Evidence of transient infection; clearance and re-infection
- SS not associated with small subset of animals



Conclusions

- Feed/diet has a profound impact on gut microbiota
- Some progress with associating microbial functional characteristics and dietary/physiological changes
- Environmental (dietary)/community (pathogen) disturbance models of microbiome state transition
- Cross-sectional studies helpful for generating prevalence data
- Longitudinal sampling and culture(-independent) studies required to:
 - Identify associations/functional interactions between NZ STEC and other members of ruminant microbial community
 - Identify efficacy of potential interventions



Acknowledgements

AgResearch Rumen Microbiology Team, Grasslands.

AgResearch, Food Assurance & Meat Quality Team, Hopkirk Institute.


mEpiLab, Hopkirk Institute, Massey University.

ESR for provision of human clinical STEC isolates

Tom Besser, Washington State University, SBI typing

Funding from NZAGRC and PGgRc, MPI and AgResearch CORE funding.



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