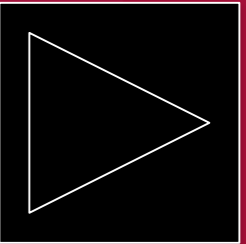




Occurrence of antibiotic residues and antimicrobial resistance genes associated with microbiome diversity in pasture-raised broilers, layers, swine, and beef cattle manures

B. R. Min¹, L. Castleberry¹, M. Rothrock², L. L. Rutherford², D. Parker¹, H. Waldrip¹, D. Brauer¹, D. W. Pitt³, and N. Indugu³

¹Conservation and Production Research Laboratory, USDA/ARS/CPRL, Bushland, TX; ²USDA-ARS; National Poultry Research Center, Athens, GA 30605; ³University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA 16802



Introduction

1. Animal manure can be a source of antibiotic resistant genes (**ARG**) and pharmaceutical residues; however, few studies have been conducted to evaluate the presence of ARG in pastured livestock systems.
2. Pasture-raised (grazing and pasture-based) livestock systems are a sustainable agricultural method for the production of layer hens, broiler chickens, other poultry, swine, beef cattle and dairy cattle, as opposed to indoor confinement facilities.
3. Despite the benefits of pastured systems, little research has focused on the environmental risk associated with grazing livestock. It is important to investigate the differences between species and farm management practices on fecal nutrient profiles, antibiotic residues (**AR**), and fecal microbiome community diversity (**MCD**) associated with ARGs.
4. The objective was to determine the effects of four pastured livestock species (broiler and layer chickens, swine, and beef cattle) from three different farms on fecal AR, MCD, and ARGs.

Methods

- Fecal DNA was collected from manure samples (25 g each WW), homogenized, and extracted (0.2 g) using a Fecal/Soil Microbe DNA MiniPrep Kit.
- Total 16S rRNA abundances, an estimate of the total MCD, were sequenced. Sulfonamide (Sul; *Sul1*) and tetracycline (Tet; *TetA*) ARGs were enumerated by qPCR.

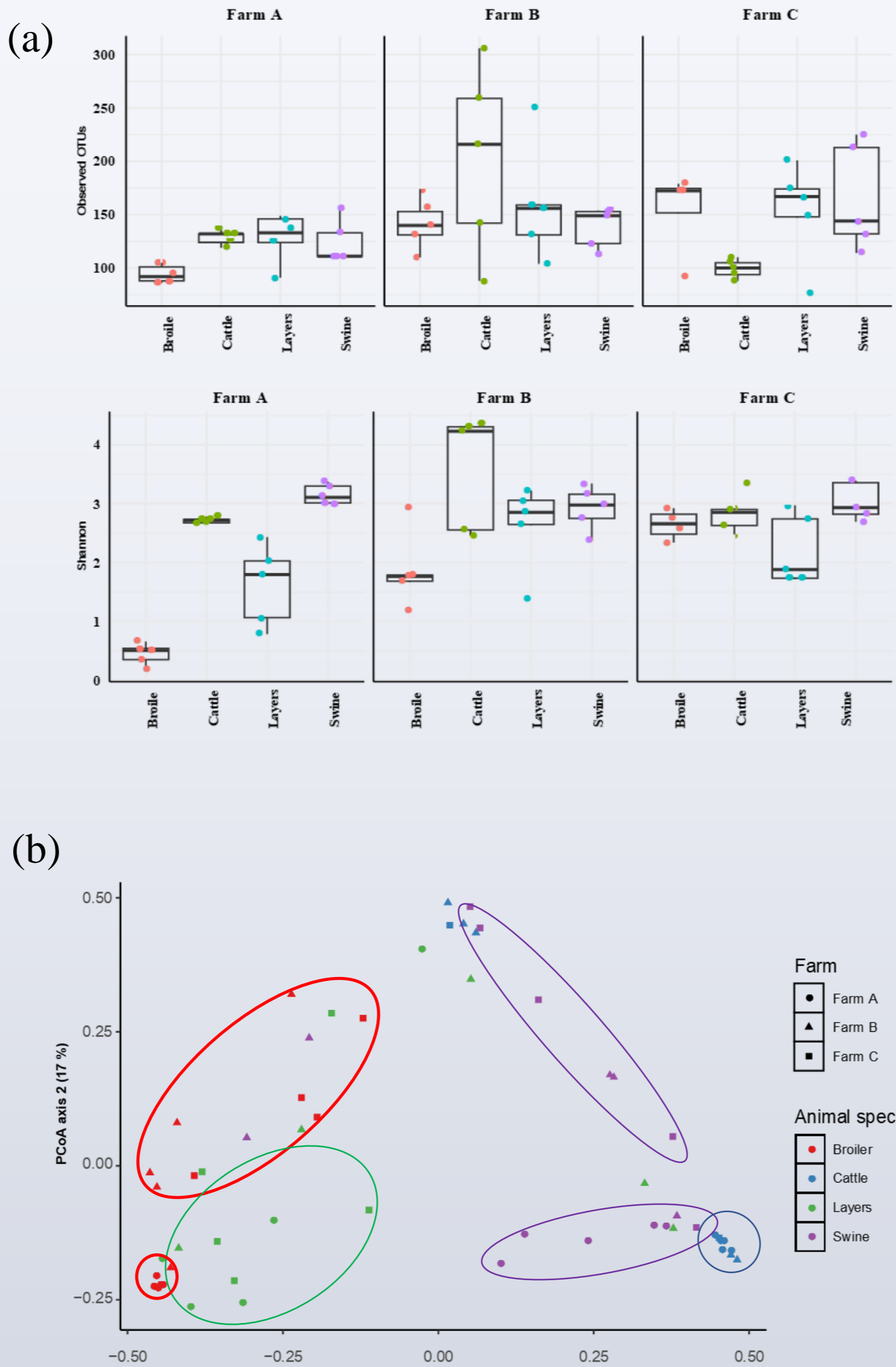


Fig 1. Alpha (a) and β -diversities (b) within and between each farm and livestock species in bacterial community compositions in broiler chickens, cattle, layer hens, and swine fed mixed pasture-based diets. Richness and diversity were measured by operational taxonomic units (OTU) and Shannon index, respectively. *P* value: farm *P* < 0.01; animal species *P* < 0.01.

Results

- The results indicated that average fecal Tet AR tended to be greater (*P*=0.09) for broiler chickens (11.4 ug/kg) than for other species (1.8-0.06 ug/kg), while chlortetracycline, lincomycine, oxytetracycline, and sulfachloropyridazine AR were similar among animal types.
- There were interactions (*P*<0.01) among farms and animal types for fecal pH and concentrations of total carbon, magnesium, sulfur, copper, and lead across animal species.
- Cattle and swine samples showed a high degree of variability (alpha- and beta-diversities) in fecal MCD, compared to broiler chicken and layer hen fecal samples (*P*<0.01; Fig. 1 a, b).
- Firmicutes (Table 1) were the most abundant bacterial phylum in feces from broiler chicken (85.3%) and layer hens (80.0%), as compared to swine (64.4%) and cattle (62.1%).
- The *Sul1* (*P*<0.05) and *TetA* (*P*<0.001) ARGs in layer hens were the highest (16.5 x E⁻⁴ and 1.4 x E⁻⁴), followed by broiler chickens (2.9 x E⁻⁴ and 1.7 x E⁻⁴), swine (0.22 x E⁻⁴ and 0.20 x E⁻⁴), and beef cattle (0.19 x E⁻⁴ and 0.02 x E⁻⁴).
- Animal types (*R*² = -0.40; *P* < 0.001), fecal pH (*R*² = 0.63; *P* < 0.001), ABR (chlortetracycline, lincomycine, and tetracycline, *R*² = 0.2 to 0.28; *P* < 0.01), total C (*R*² = -0.33; *P* < 0.001), total N (*R*² = -0.48; *P* < 0.001), C/N ratio (*R*² = 0.41; *P* < 0.001), and the most of major mineral contents (Ca, P, K, Mn, Na, and Zn; *R*² = -0.27 to -0.59; *P* < 0.01 to 0.001) were positively or negatively correlated with *TetA* ARG.

Table 1. Bacterial phylum profiles and antimicrobial resistance genes (ARG) for the four domesticated animal metagenome samples.

Average	Farm					Livestock					
	A	B	C	SEM	P-value	B	L	S	C	SEM	P-value
DNA concentration, (ng μ L ⁻¹)	28.5 ^a	25.8 ^{ab}	18.2 ^b	2.82	0.01	21.3	24.2	29.3	21.9	3.76	0.014
Bacterial phylum											
Firmicutes (F)	82.4 ^a	61.1 ^c	75.3 ^b	2.29	0.01	85.3 ^a	80.0 ^b	64.4 ^b	62.1 ^b	3.06	0.01
Bacteroidetes (B)	10.1	14.0	11.6	1.69	0.18	2.4 ^c	5.7 ^c	16.5 ^a	23.0 ^a	2.28	0.001
Actinobacteria	0.8 ^b	9.0 ^b	2.2 ^a	1.30	0.01	3.6 ^{ab}	2.4 ^b	7.1 ^a	3.0 ^{ab}	1.73	0.05
Proteobacteria	3.0 ^b	11.7 ^a	7.8 ^{ab}	1.84	0.01	8.3	8.4	6.2	7.2	2.46	0.53
F/B ratio ¹	0.9 ^a	0.7 ^b	0.8 ^{ab}	0.05	0.01	1.0 ^a	0.9 ^a	0.7 ^b	0.6 ^b	0.05	0.01
Antimicrobial resistance genes (ARG)											
<i>Sul1</i>	0.4 ^b	1.4 ^a	0.3 ^b	0.41	0.05	2.9 ^b	16.5 ^a	0.22 ^b	0.19 ^b	0.552	0.04
<i>TetA</i>	1.1 ^a	1.1 ^a	0.4 ^b	0.24	0.02	1.7 ^a	1.4 ^a	0.2 ^b	0.02 ^b	0.32	0.001

Conclusions

- Animal production for human consumption or use is a source of environmental ARGs; however, the relatively high ARG concentrations found in intensive livestock farms are not present to the same extent in manure from pastured animals.
- Enhancing the quantitative ARG database is critical to improve risk assessment and to develop and validate models that estimate how antibiotic use in animal production systems can affect ARG abundance in receiving environments and clinical settings.
- This study concluded that individual farm management practices and specific animal types influenced concentrations of fecal AR, the fecal MCD, and ARG status in pasture-raised animals.