Increased microbial diversity and decreased prevalence of common pathogens in the gut microbiomes of wild turkeys compared to domestic turkeys

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Abstract

Turkeys (*Meleagris gallopavo*) provide a globally important source of protein and constitute the second most important source of poultry meat in the world. Bacterial diseases are common in commercial poultry production causing significant production losses for farmers. Due to the increasingly recognized problems associated with large-scale/indiscriminant antibiotic use in agricultural settings, poultry producers need alternative methods to control common bacterial pathogens. In this study we compared the cecal microbiota of wild and domestic turkeys, hypothesizing that environmental pressures faced by wild birds may select for a disease-resistant microbial community. Sequence analysis of 16S rRNA genes amplified from cecal samples indicate that free-roaming wild turkeys carry a rich and variable microbiota compared to domestic turkeys raised on large-scale poultry farms. Wild turkeys also had very low levels of *Staphylococcus, Salmonella* and *E. coli* when compared to domestic turkeys. *E. coli* strains isolated from wild or domestic turkey cecal samples also belong to distinct phylogenetic backgrounds and differ in their propensity to carry virulence genes. *E. coli* strains isolated from factory-raised turkeys were far more likely to carry genes for capsule (*kpsII*, *kpsIII*) or siderophore (*iroN*, *fyuA*) synthesis than those isolated from wild

turkeys. These results suggest that the microbiota of wild turkeys may provide colonization resistance against common poultry pathogens.

Importance

Due to the increasingly recognized problems associated with antibiotic use in agricultural settings, poultry producers need alternative methods to control common bacterial pathogens. In this study we compare the microbiota of wild and domestic turkeys. Results suggest that free ranging wild turkeys carry a distinct microbiome when compared to farm raised turkeys. The microbiome of wild birds contains very low levels of poultry pathogens compared to farm raised birds. The microbiomes of wild turkeys may be used to guide development of new ways to control disease in large scale poultry production.

Introduction

Turkeys (*Meleagris gallopavo*) evolved approximately 11 million years ago and are one of the first birds domesticated in the Americas (1-3). Although domesticated thousands of years ago, turkeys have remained generally very similar to their wild relatives until relatively recently (4, 5). In the past ~70 years, intensive selective breeding of turkeys has resulted in dramatic changes in commercially raised birds compared to their wild relatives, leading to a genome that is much less diverse than many other agricultural species (4). These genetic changes as well as advancements in production practices have resulted in domestic birds maturing much more quickly and reaching three times the body mass of wild birds at maturity (6). Domestic turkeys are now the second most important source of poultry in the world, with the USA producing ~250,000,000 turkeys and ~7,000,000,000 pounds of turkey meat in 2019 (7).

Relatively few studies have been published comparing the microbiomes of wild animals and their domesticated kin. However, the limited literature on this topic has overwhelmingly shown that the microbiome of captive and wild animals varies dramatically (8-15). The observed differences in microbial communities between wild and captive animals has led for calls for more research on the microbiomes of additional wild animals (16, 17).

The gut microbiome of poultry is known to contribute to efficient growth as well as bird health (11, 18-21). The microbiome of commercially raised poultry is undoubtedly influenced by production practices such as crowded conditions, diet, and antibiotic use. Several studies have characterized the gut microbiomes of domestic turkeys in a variety of experimental and agricultural settings (20, 22-25); however very few studies have focused on the microbiomes of wild turkeys (11).

In an effort to better characterize potential effects of gut microbiota on turkey health and disease, we compared the cecal microbiota from factory-raised domestic, free-ranging domestic and free-ranging wild turkeys. Sequencing of the V4 region of 16S DNA was used to determine the abundance of multiple taxa in the ceca of individual birds within each group. Additional experiments were designed to determine the prevalence of bacterial taxa which are common pathogens of commercially raised turkeys. These studies indicate that beta diversity within the microbiota is significantly different between factory-raised domestic turkeys, free-ranging domestic turkeys and free-ranging wild turkeys. Several common pathogens associated with commercial poultry production (*E. coli*, *Salmonella and Staphylococcus sp)*, were infrequent or absent in the cecal microbiota of free-ranging wild turkeys. *E. coli* strains found in wild turkeys were found to be genetically diverse and carry fewer virulence associated genes than strains found in factory-raised birds.

Materials and Methods.

Definition of turkey groups used in this study

The term “wild turkey” can mean both a strain of turkey, as well as the lack of domestication. In this study, we define “wild turkey” as a population of self-sustaining, wild, free-ranging birds. All wild turkeys sampled in this work were of the Rio Grande subspecies (*Meleagris gallopavo intermedia*), that have ranged freely for generations in the mountains of North Central Utah, USA. Birds described as “free-range domestic turkeys” in this study are domesticated turkeys ranging freely outdoors. All free-range domestic turkeys in this study were from hobby farms where they were allowed to

forage freely outdoors both summer and winter. The diet of all domestic free-range turkeys was supplemented with commercial poultry food by their owners. The term "factory-raised domestic turkey" refers to turkeys raised in commercial turkey production facilities. All turkeys in this group were of the *Broad Breasted White* variety. Although these factory-raised birds may fit the legal definition of "free-range" by virtue of their caging conditions, they were not considered "free-ranging" for the purposes of this study.

Collection of cecal samples

Some birds, including turkeys and chickens, produce two distinctly different types of feces. Cecal drops are a type of feces that the bird periodically excretes directly from the intestinal cecum (26). Previous work has demonstrated that the ceca contains the greatest microbial diversity found in the intestinal tract of poultry (27, 28). Additionally, the microbiota found in cecal drops is highly reflective of the microbiota found in cecal contents collected following sacrifice of the bird (29). The collection of cecal drops, which are easily distinguishable from normal feces, enables a simple, noninvasive method of obtaining a clear view of the cecal microbiota and eliminates the need to sacrifice (or even come in contact with) study animals.

In this study all samples were of cecal origin. Cecal drops from wild and free-ranging domestic turkeys were collected during winter months following snowstorms. Sample collection immediately following snowstorms ensured that only fresh samples were collected and the sample remained relatively uncontaminated by bacteria from the soil or other environmental sources. Cecal contents from one flock of factory-raised

turkeys were collected from a turkey processing facility post mortem. Cecal drops from a second commercially raised flock were collected from the floor of the production facility. Sampling sites, bird age and other details of sample origin are listed in Supplementary Data S1

DNA preparation

Following sample collection, all cecal contents were kept frozen until DNA isolation. DNA used for V4 sequencing was extracted from each sample using the Zymo *Quick*-DNA Fecal/Soil Microbe 96 Kit (Zymo D6011) according to the manufacturer's instructions, including a bead homogenization step using a 2010 Geno/Grinder (Spex, Metuchen, NJ) at 1750 RPM for 10 min. DNA was prepared for 16S rRNA gene V4 region sequencing based on an established protocol with minor deviations (30). First, the V4 region of the 16S rRNA gene was amplified individually from each sample with the AccuPrime Pfx Enzyme (ThermoFisher Scientific, Waltham, MA, USA) in 20 μl volumes using a subset of the exact primer sequences described previously (30). PCR amplicons were normalized using the SequalPrep Normalization kit (Applied Biosystems, Waltham, MA, United States), pooled in groups of 96 reactions, and fragments in the range of 250-450 bp were purified using a BluePippin (Sage Science, Beverly, MA) selection step. Equimolar normalization of each pool and sequencing was performed at the BYU DNA Sequencing center on a partial 2 x 250 lane (v2) of a HiSeq 2500 (Illumina, Inc., San Diego, CA). Laser complexity was assured by including at least 10% of each lane with shotgun sequencing libraries for other bacterial genomes.

Sequences were deposited to the National Center for Biotechnological Information Short Read Archive as (accession forthcoming).

Sequence Analysis

Sample reads were demultiplexed on the Illumina platform and analyzed using QIIME2 (31, 32) and R. briefly, reads were trimmed to maximize quality scores of each nucleotide position. DADA2 (33) was used to denoise, dereplicate, and call amplicon sequence variants (ASVs), taxonomy was assigned to the ASVs using the GreenGenes classifier 13_8_99 (34). ASV tables were filtered to 13,000 reads per sample and differences between groups were determined by PERMANOVA (35) of weighted and unweighted Unifrac distances (36, 37). To permit calculating Unifrac distances we built a phylogenetic tree with fasttree2 (38) based on mafft alignment (39). Differences in OTU abundances between samples were performed using ANCOM (40). Abundances of individual OUTs were manually analyzed based on the taxonomic assignments, which were assigned to OTUs using the QIIME2 q2-feature-classifier (41). Alpha diversity metrics were defined using QIIME2 and differences in alpha diversity metrics between sampling locations was determined by a Kruskal-Wallis test.

Determination of relative *E. coli* DNA levels in cecal samples

A qPCR based assay was designed to estimate the relative abundance of *E. coli* DNA in each cecal sample, based on detection of the *ybbW* gene, which is found exclusively in *E. coli* (42). Primer probe sets and reaction conditions can be found in Supplemental Data (Figure S2). The efficiency and reproducibility of amplification was

verified by generating a standard curve using doubling dilutions of positive control DNA. Negative controls consisted of reaction mixtures with DNA elution buffer rather than DNA. Each sample was tested in duplicate. Purified DNA from pooled *E. coli* strains was used as a positive control.

Isolation and Genotyping of *E. coli* strains

E. coli present in cecal samples were isolated by homogenizing a portion of the sample in sterile PBS and plating on MacConkey agar, followed by growth at 37 ºC for 24 h. Colonies with characteristic *E. coli* morphology were then restreaked and verified as *E. coli* by PCR targeting the *ybbW* gene. Total DNA was isolated from individual colonies using mini-genomic DNA kit for blood and cultured cells (IBI Scientific). Putative *E. coli* strains were assigned to phylo-groups using the Clermont quadriplex assay, with additional PCR tests to distinguish group C or group E when warranted, as previously described (43). Presence or absence of genes associated with virulence of avian pathogenic *E. coli* (*iutA*, *iss*, *iroN, fyuA, kpsMTII, kpsMTIII*) was determined by PCR, using primers contained in Supplemental Data S2.

Detection of *Salmonella* DNA in cecal samples

Presence or absence of *Salmonella* sp. in cecal sample DNA was determined using a semi-quantitative PCR assay based on detection of the *invA* gene, which has previously been demonstrated to specifically detect most *Salmonella* strains (44). Primer sequences and PCR conditions are outlined in Supplemental Data S2.

Results

To explore potential differences in the microbiota of wild vs domestic turkeys, a 16S rRNA gene survey of the cecal microbiota was performed. A total of 4,070,891 bacterial reads were obtained, with an average of 53,564 reads per sample and 3,069 ASVs. We performed principal coordinate analysis (PCoA) and PERMANOVA of weighted Unifrac distances to compare the microbiota composition of different flocks of turkeys. At a 13,000-read subsampling depth PERMANOVA of Unifrac distances revealed significant differences in the microbiota of the samples within provenance and flock (Table 1, Supplementary Data S3). The clustering of samples on PCoA ordinations visually depicted these statistical differences, where Principal coordinates 1 and 2 separated the samples into three general groups that matched the provenance of the samples when analyzed by both weighted and unweighted Unifrac distance (Figure 1, Table 1). Follow up weighted and unweighted Unifrac analyses confirmed there were flock-specific effects when each provenance was analyzed separately, except for birds from factory-raised flocks, analyzed by weighted Unifrac (Table 1). The finding that all flocks differed in beta diversity, except those raised in commercial production facilities, is likely a reflection of the highly standardized nature of commercial poultry production.

We also evaluated the variation in the microbiota composition of the different flocks. At the order level there were significant differences in the numerical density of the most abundant bacterial taxa (Figure 2A). For example, in the factory-raised birds, *Clostridiales* was the most abundant taxon (71.7% +/- 3.3%), much more than in free-ranging domestic turkeys (33.8% +/- 1.8%) or wild turkeys (18.3% +/- 0.7%). The lower *Clostridiales* read counts in the free-ranging domestic and wild turkeys were largely

offset by relative increases in *Bacteroidales* and *Coriobacteriales*. The abundances of these reads were all significantly different between provenances by ANCOM (Supplementary Data S4). However, despite these differences in abundance of different taxa, variation in alpha diversity between flocks was not related to the flocks' provenance (Table 2). Therefore, key differences in numerical composition at high taxonomic levels did not necessarily reflect low-level differences in diversity.

To better understand the potential relationship between flock provenance and carriage of potential pathogens, we next focused on the relative abundance of taxa known to be of veterinary and medical importance by identifying ASVs that best matched known bird pathogens. V4 sequences representing *Staphylococcus sp*. were most prevalent in samples from commercially raised birds. *Staphylococcus* DNA was also detected in one flock of domestic free-ranging turkeys. Detectable levels of *Staphylococcus* DNA were not found in any samples from wild birds. Similarly, *Campylobacter* DNA was identified only in factory-farm raised birds. The abundance of *Campylobacter* DNA in some birds was suggestive of heavy colonization; however, it was undetected in other birds with in the same flock (Figure 2B, C,).

One limitation of our approach is that without whole-genome data, the short region we sequenced cannot distinguish known pathogens from similar bacteria with identical sequences across the 16S V4 region. Measurable levels of the family *Enterobacteriaceae* were abundant in samples from both factory-raised flocks and one free-ranging domestic flock (Figure 2D). The *Enterobacteriaceae* are a large family of bacteria that include *Escherichia coli* as well as other pathogens including *Salmonell*a. The V4 region of the 16S rRNA gene does not resolve *E. coli* or *Salmonella* from other

Enterobacteriaceae, which prevented us from estimating *E. coli* or *Salmonella* abundance in these animals through V4 sequencing alone. As *E. coli* and *Salmonell*a are common pathogens in domestic poultry production, we further investigated the prevalence of these potential pathogens in wild and factory-raised turkeys.

The presence of *Salmonella* DNA was detected by PCR targeting the *Salmonella* specific gene *invA* in total DNA isolated from cecal samples of individual birds. Of 14 samples tested from factory-raised birds, 11 tested positive for *invA*. Conversely, none of 11 samples collected from wild birds tested positive for the presence of the *invA* gene, suggesting that factory-raised turkeys more frequently contain *Salmonella* in their digestive tracts than wild turkeys. To determine the relative abundance of *E. coli* in cecal samples, a qPCR assay targeting the *E. coli* specific gene *ybbW* (42) was used. Genomic *E. coli* DNA was clearly present in the total DNA samples obtained from commercially-raised turkeys. Conversely, *E. coli* DNA in samples from wild turkeys was below the limit of detection of the assay (Figure 3). We also plated cecal samples on MacConkey agar to enrich for growth of enteric bacteria. Although not detectable by qPCR, we were able to isolate colonies characteristic of *E. coli* from wild turkey cecal samples. Their identity as *E. coli* was subsequently verified by amplification of the *ybbW* gene. *E. coli* were readily cultured from the ceca of factory-raised turkeys. In addition to colony growth consistent with *E. coli* (pink colonies), white colonies were also observed growing on MacConkey agar. These white colonies were not studied further or collected; however, based on growth on MacConkey agar, these colonies were likely *Salmonella* or other enteric bacteria.

As we were able to isolate *E. coli* colonies from the ceca of both wild and factory-raised turkeys, we were interested in further understanding the differences that may exist between these bacterial populations. We therefor performed phylo-group analysis to compare the diversity of *E. coli* lineages that were isolated from factory-raised and wild turkeys. Of *E. coli* isolated from wild turkeys, 29 of 30 strains belonged to groups A, B1, or E whereas none belonged to groups B2, C, or D (Figure 4 and Supplementary Data S5). Strains isolated from factory-raised turkeys were more diverse with all major phylo-groups represented. Several (9/50) belonged to cryptic clades I or II, which have been infrequently isolated in other studies. Seven strains were classified as group B2 or D, which are lineages that are commonly associated with extraintestinal pathogenic *E. coli* strains (45, 46). These results suggested that the pathogenic potential of the *E. coli* present in wild turkeys may be different from those present in factory-raised domestic turkeys.

A number of virulence factors have been identified in extraintestinal pathogenic *E. coli*. These include proteins essential for iron acquisition and for group 2 or group 3 capsule production (47-53). To determine if virulence associated gene carriage differed between *E. coli* found in wild turkeys and factory-raised turkeys, end point PCR was used to determine carriage of three siderophore receptor genes (*iutA, iroN,* and *fyuA*) as well as the *kpsMT* genes involved in group 2 or group 3 capsule synthesis (Table 3). Nearly half (47%) of *E. coli* strains isolated from wild turkeys carried the aerobactin receptor *iutA* gene. However, carriage of the salmochelin receptor *iroN*, yersiniabactin receptor *fyuA*, or capsule synthesis *kpsMT* genes was not observed in *E. coli* strains isolated from wild turkeys. Conversely, only 10% of strains isolated from the ceca of

commercially produced turkeys contained *iutA*, whereas *iroN* was present in 22% and *fyuA* in 4%. Capsule synthesis genes (*kpsMTII* or *kpsMTIII*) were present in 40% of strains isolated from factory-raised turkeys. Presence of virulence factors was not associated with any particular phylo-group, and several strains carried combinations of virulence factor genes (Supplemental Data S5).

Discussion

The essential role of gut microbiota in maintaining animal and human health has been well established (54-57). Although diet is clearly an important selector for many functional guilds of microbes within the gut, host evolutionary history is thought to be a driving factor in determining the prevalence of specific microbial OTUs (58). Increasingly, evidence supports the theory that many animals coevolved with their microbial symbionts giving both host and microbe survival advantages (59-62).
In addition to diet, the intestinal microbiome of domestic farm animals (including poultry) is likely influenced by a number of factors, such as, past and present exposure to antibiotics, exposure to the microbiome of the mother and other microbes in their environment. Data presented here suggest that common production practices (potentially in combination with) selective breeding in modern poultry farming have resulted in a turkey microbiome in which beta diversity decreases from wild birds to free-ranging domestic birds to the highly monotaxic microbiota seen in commercially raised turkeys. V4 sequencing results from this study are largely consistent with a previous clone-based sequencing approach comparing the microbiota of wild turkeys and domestic turkeys (11).

The composition of the gut microbiota in poultry likely influences a variety of beneficial characteristics, including immune system development and function (63-65). Domestic turkeys, raised in commercial turkey production facilities are highly susceptible to a myriad of economically devastating bacterial, fungal, viral and parasitic diseases (50). Previous research has shown that colonization by some commensal species of microbes prevents/inhibits colonization by pathogenic *Campylobacter, Staphylococcus* and *Salmonella* (66-73) in poultry. We hypothesize that wild relatives of agriculturally important species may carry a heritable microbiome which inhibits colonization by common pathogens. Modern agricultural production practices have largely ignored the potential benefits of this natural microbiota, having instead relied on widespread use of antibiotics to control pathogens.

While we have not yet established any specific mechanistic links between members of the normal flora and the abundance of specific pathogenic species, we observed that wild turkeys have higher levels of Coriobacteriales compared to hobby farm or factory-raised domestic turkeys. Some Coriobacteriales produce hydroxysteroid dehydrogenase enzymes involved in the conversion of primary to secondary bile acids (74, 75). Bile salt conversion has demonstrated effects on composition of the microbiome, colonization of intestinal pathogens, and immune responses in humans and livestock (74, 76-79). Growth of Coriobacteriales is stimulated by polyphenols found in diverse plants, and these bacteria metabolize them to phenolic compounds that have anti-inflammatory and immunomodulatory effects (75). Coriobacteriales are also especially prone to disruption by antibiotic treatment in mice (80). The diets of wild turkeys are free from agricultural antibiotics and likely contain diverse plant polyphenols.

Whether members of this family are involved in colonization resistance to *Campylobacter*, *Salmonella*, or *E. coli* should be investigated further.

Suppression of avian-pathogenic *E. coli* in turkeys is an especially important priority for poultry producers. Therefore, it is notable that wild turkeys appeared to contain very little *E. coli* in their ceca. The *E. coli* strains isolated from wild turkeys were dissimilar to those isolated from factory-raised birds, both in terms of phylogenetic lineage as well as the presence of specific virulence factors. Many of the *E. coli* strains isolated from wild turkeys contained the aerobactin receptor gene. Aerobactin is a proven virulence factor in extraintestinal avian infections (51); however its role in these strains may be related to fitness in the highly competitive environment of the wild turkey intestinal tract. The absence of capsule synthesis genes, salmochelin, and yersiniabactin production from strains isolated from wild turkeys may indicate that these strains are not prone to cause bloodstream infections or colonize other organs. It is possible that bacteriocins, prophages, contact-dependent inhibition or type 6 secretion systems of *E. coli* lineages established wild turkeys exclude invasion by avian-pathogenic strains frequently found in factory-raised poultry (81-84).

Due to common poultry production practices, microbes colonizing the intestinal tract of commercially raised poultry are minimally, if at all, influenced by the microbiome of the mother. The practice of hatching surface sterilized eggs in incubators for multiple generations has surely contributed to the loss of heritable microbial taxa which coevolved with the wild turkey over millennia. As a consequence, modern production practices have likely resulted in domestic poultry obtaining their microbiota almost exclusively from the environment found in the production facilities in which they are

raised. These conditions have likely skewed the gut microbiota toward taxa best capable of survival in modern turkey production facilities rather than taxa contributing to the mutual survival of host and microbe.

The transfer of microbiota from mother to infant has been best characterized in mammals. The transfer of maternal microbes to the mammalian young begins during the birthing process and continues through nursing and social interactions (85, 86). Coprophagy is common in many animal species, including turkeys (87-89) and it is common to see turkeys consuming cecal drops of their cage mates. This innate behavior in turkeys may have evolved to enable bird-to-bird spread of beneficial microbiota within a flock. Recent work documents that the newly hatched young of some birds readily consume cecal drops, but not normal rectal feces, of their mothers. This consumption of maternal cecal drops by chicks was observed only during a short window of time (approximately the first month of life) (90). This behavior potentially facilitates the establishment of a beneficial, heritable gut microbiome from mother to chick.

The gut microbiome is perhaps one of the most complex of biological communities. As in the analysis of any biological community, it is essential to consider the effects of dominant taxa, as well as taxa that may comprise a relatively small, but potentially important, role in the community as a whole. The goal of this study was to identify potential changes/differences in the microbial composition of factory-raised turkeys when compared to their wild predecessors. Results presented here demonstrate that the overall abundance of *E. coli*, *Salmonella, Campylobacter* and *Staphylococcus* in wild turkeys is much lower than levels commonly found in

commercially raised turkeys. Furthermore, *E. coli* strains occupying the intestinal tract of wild turkeys appear distinct from strains commonly found in commercially raised turkeys. The strong correlation between bird provenance, increased microbial diversity and low pathogen carriage warrants further research into the potential for mining the microbiome of free-ranging wild turkeys (as well as wild relatives of other agriculturally important species) in search of therapeutics or probiotics for use in controlling pathogens common in agricultural food production.

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Figures and Tables

Figure 1

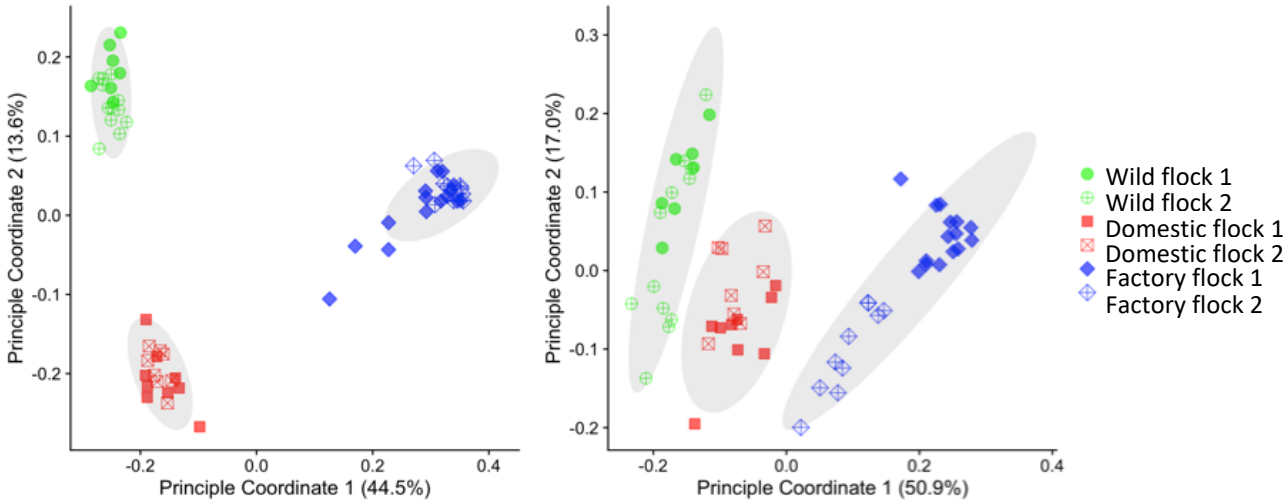

Figure 1. Principal Component Analysis demonstrates the cecal microbiota of turkeys' cluster according to bird provenance. Weighted (A) and unweighted (B) Unifrac distance plots of the different flocks, colored according to the animals' provenance.

Figure 2A

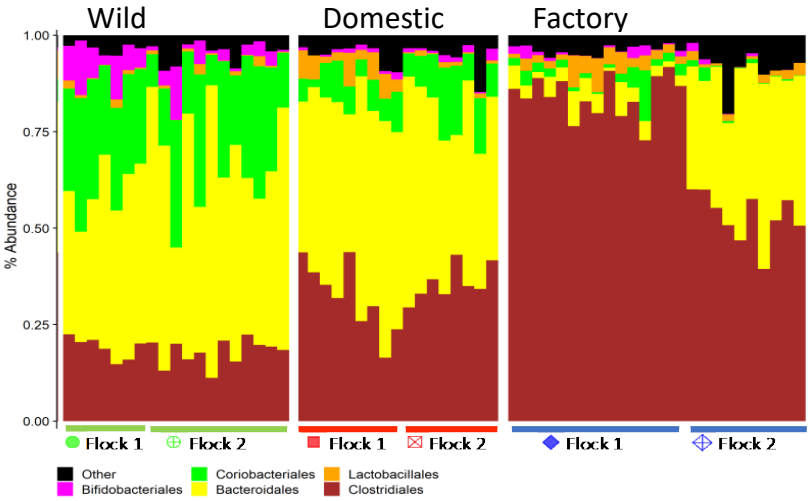

Figure 2A. Taxa abundance differs widely between wild and factory raised domestic turkeys. Taxon plot of flocks, grouped by provenance. Order level assignments above 2% total relative abundance are shown individually.

Figures 2B, C, D

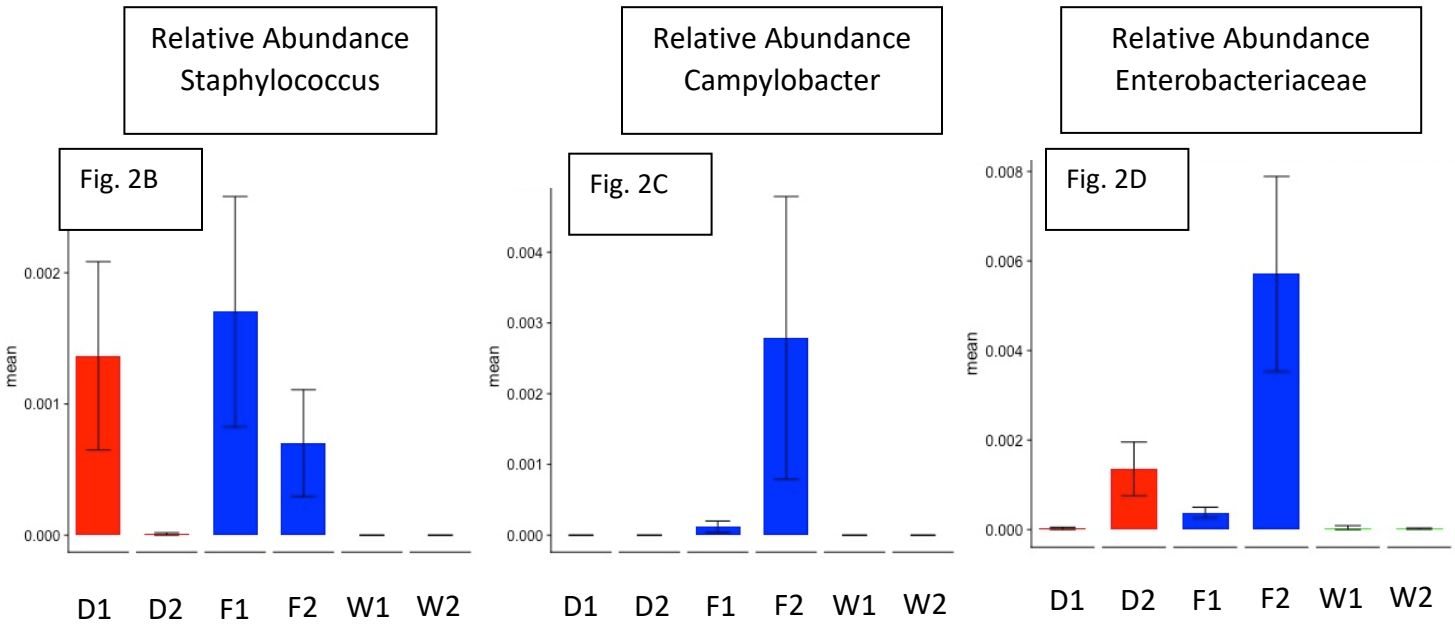

Figure 2B, C, D. *Staphylococcus*, *Campylobacter* and Enterobacteriaceae are more abundant in the cecal contents of factory raised domestic turkeys than wild turkeys. Relative abundance of groups of ASVs (B, C) or an individual ASV (D) from the 16S sequencing dataset. Samples are grouped according to flock and colored by provenance: Red (D) = domestic birds raised on hobby farms; Blue (F) = Domestic birds raised in factory farms; Green (W) = free ranging wild turkeys)

Figure 3

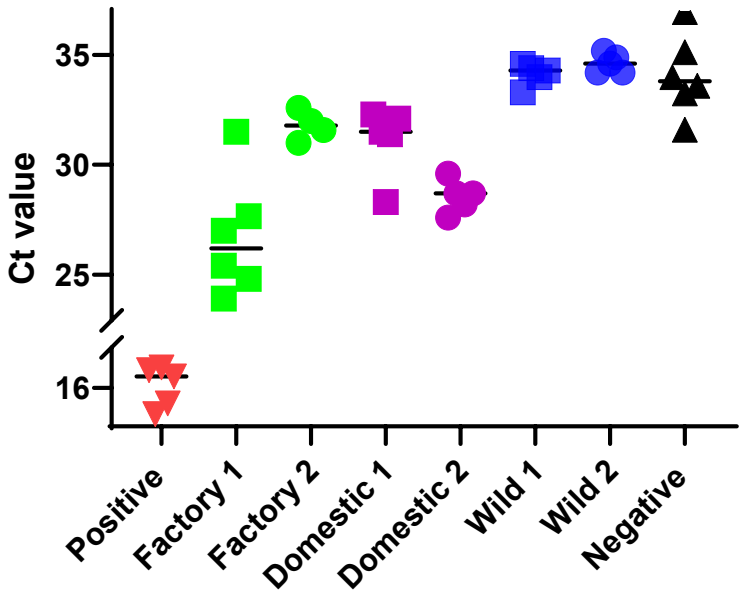

Figure 3. Cecal contents of factory raised and free ranging domestic turkeys contain high levels of *E. coli* DNA compared to wild birds. Quantitative PCR for the *ybbW* gene (*E. coli* specific) detected in total genomic DNA isolated from the ceca of factory-raised domestic, free-range domestic, and wild turkeys. CT values from individual birds are shown in the Y-axis. Average Ct values of each group are indicated by a horizontal bar. Individual flocks, positive control (100% *E. coli* genomic DNA) and negative control are indicated in the X-axis.

Figure 4

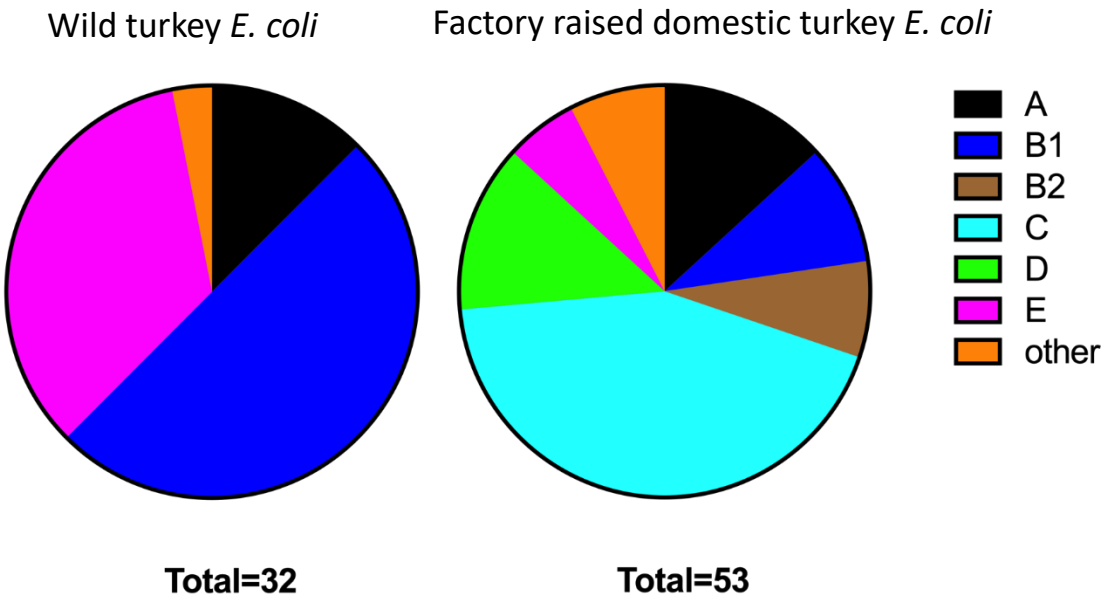

Figure 4. *E. coli* strains isolated from wild turkeys are predominantly phylo-groups B1 and E. A wide variety of phylo-groups populate the intestinal tract of factory raised turkeys Phylo-groups represented by color as indicated.

Table 1

Table 1. PERMANOVA tables for different groups of samples. Df = degrees of freedom, SS = sum of squares, MS = mean of squares, F = F statistics, R^2 = R^2 value. P = p-value.

	df	SS	MS	F	R^2	p	SS	MS	F	R^2	p
		Weighted Unifrac					Unweighted Unifrac				
ALL SAMPLES											
Provenance	2	1.68	0.84	55.57	0.55	<0.001	5.12	2.56	44.99	0.56	<0.001
Flock	3	0.52	0.17	11.38	0.17	<0.001	0.82	0.27	4.79	0.09	<0.001
Residuals	55	0.83	0.02		0.27		3.13	0.06		0.35	
Total	60	3.03			1.00		9.06			1.00	
WILD											
Flock	1	0.08	0.08	5.08	0.25	<0.001	0.27	0.27	5.02	0.25	<0.001
Residuals	15	0.22	0.01		0.75		0.81	0.05		0.75	
Total	16	0.30			1.00		1.08			1.00	
DOMESTIC											
Flock	1	0.38	0.38	38.71	0.63	<0.001	0.35	0.35	5.20	0.18	<0.001
Residuals	23	0.23	0.01		0.37		1.53	0.07		0.82	
Total	24	0.61			1.00		1.88			1.00	
FACTORY											
Flock	1	0.06	0.06	2.67	0.14	0.074	0.20	0.20	4.34	0.20	<0.001
Residuals	17	0.38	0.02		0.86		0.79	0.05		0.80	
Total	18	0.44			1.00		0.99			1.00	

Table 2.

Flock	Shannon	Pielou's Evenness	Faith's PD	Observed ASVs
Wild flock 1	6.03 +/- 0.09 [ab]	0.753 +/- 0.009 [ab]	17.2 +/- 0.9 [a]	260 +/- 13 [ab]
Wild flock 2	6.43 +/- 0.10 [cd]	0.77 +/- 0.008 [ac]	21.2 +/- 0.7 [b]	330 +/- 18 [c]
Domestic flock 1	6.61 +/- 0.12 [ac]	0.785 +/- 0.010 [a]	21.1 +/- 0.9 [b]	347 +/- 21 [ad]
Domestic flock 2	6.96 +/- 0.03 [ac]	0.81 +/- 0.007 [a]	22.4 +/- 0.8 [a]	402 +/- 20 [ad]
Factory flock 1	6.35 +/- 0.14 [d]	0.76 +/- 0.012 [c]	16.4 +/- 0.7 [b]	330 +/- 15 [cd]
Factory flock 2	5.80 +/- 0.18 [b]	0.72 +/- 0.014 [b]	16 +/- 0.9 [a]	272 +/- 22 [b]

Table 2. Alpha diversity metrics on a per-flock basis as mean +/- s.e.m.. Different letters next to the s.e.m. represent significant differences between flocks for each metric and were determined by a Kruskal-Wallis test

Table 3

Prevalence of virulence factors genes in *E. coli* strains isolated from wild and factory raised turkeys. VF= virulence genes

Wild Turkeys	
No VF	0 of 32
iutA	15 of 32
iroN	1 of 32
fyuA	0 of 32
kpsII	0 of 32
kpsIII	0 of 32
2 VF	0 of 32
3 VF	0 of 32
4 VF	0 of 32

Factory Raised Turkeys	
No VF	15 of 53
iutA	5 of 53
iroN	13 of 53
fyuA	7 of 53
kpsII	23 of 53
kpsIII	4 of 53
2 VF	9 of 53
3 VF	1 of 53
4 VF	1 of 53

Supplemental Data Figure S1

Code	Source	Latitude	Longitude	Strain	Age	Provenance	Diet	Habitat	Source
Domestic Flock 1	Mapleton, UT, USA	40 08N	111 33W	Midget White	~8 months	Free range domestic	Forage + commercial poultry diet	~1 acre dirt/grass	Cecal drops
Domestic Flock 2	Lindon, UT, USA	40 N	111 41W	Unknown/ Mixed	>1 year	Free range domestic	Forage + commercial poultry diet	~3 acres trees, brush grass	Cecal drops
Wild Flock 1	Payson Canyon, UT, USA	39 59 N	111 42W	*Meleagris gallopavo* subspecies Rio Grande	>6 months	Free range wild	Forage	Gambles oaks, cottonwood trees predominate	Cecal drops
Wild Flock 2	Hobble Creek Canyon, UT, USA	40 12N	111 29W	*Meleagris gallopavo* subspecies Rio Grande	>6 months	Free range wild	Forage	Gambles oaks, cottonwood trees predominate	Cecal drops
Factory Flock 1	Pitman Farms, Moroni, UT, USA	39 31N	111 35W	*Broad Breasted White*	~18 weeks	Commercially raised	Commercial diet	Commercial farm	Dissected ceca
Factory Flock 2	Sanpete County, UT, USA	39 22 N	111 35 W	*Broad Breasted White*	~6 weeks	Commercially raised	Commercial diet	Commercial farm	Cecal drops

Supplemental Data S2

qPCR

For qPCR experiments, total DNA was isolated from cecal samples using the Qiagen blood and tissue DNA kit as directed by the manufacturer. DNA samples were diluted to a concentration of 100ng/µl total DNA. IDT PrimeTime® Gene Expression Master Mix was used in all qPCR assays. PCR conditions: Briefly, thermocycling was performed as suggested by manufacture (40 cycles of 95 ºC denaturation for 15

seconds followed 57 ºC annealing/amplification for one minute) using an ABI StepOnePlus™ Real-Time PCR System. qPCR primers and probe were designed using IDT PrimerQuest® and manufactured by Integrated DNA Technologies.

Primer and probe sequences for *ybbW*

Primer 1F	TGATTGGCAAATCTGGCCG
Primer 1R	CGTTGACCAGCCAGAAGATTAAG
Probe	56-FAM/AAGCCCGGT/ZEN/AGAGAAAGGCCTAAC/3IABkFQ\

invA Detection

In endpoint PCR experiments to detect the presence of the *invA* gene, OneTaq master mix (NEB) was used in all reactions. The following PCR conditions were used: 95 ºC for 2 minutes followed by 30 cycles of 95 ºC for 20 sec. 56 ºC for 20 sec. 68 ºC for 40 sec. followed by a 7 minute final extension step at 68 ºC. Amplified DNA was visualized by running the product on a 1.75% agarose gel.

Primer sequences for *invA*

invA	Primer 1F GTGAAATTATCGCCACGTTCGGGCAA
invA	Primer 1R TCATCGCACCGTCAAAGGAACC

Phylo-Group Determination

Phylogrouping was performed as previously described (1) except that we used 10 µl reactions in OneTaq master mix (NEB) with an extension temperature of 68 degrees. We first performed the quadriplex PCR reaction, followed by group E or group C specific PCR when warranted to distinguish between D/E or A/C strains.

Virulence genotyping was performed using approximately 100 ng genomic DNA as template and 20 pmol of each primer in OneTaq master mix. The conditions were 94 ºC for 3 min followed by 30 cycles of 94 ºC for 15 seconds, 57 ºC for 15 seconds, and 68 ºC for 45 seconds, and a final extension of 68 ºC for 5 min. The *fyuA, kpsII* and *kpsIII* reactions were multiplexed, the *iutA iroN* and *iutA* reactions were run individually.

Primer Names, Sequences, Target and Expected Amplicon Size.

Primer	**Sequence**	**Target, Expected size**
iutA F	ctgcagtactccgatcggctg	iutA, 470
iutA R	tggttggaggtaaagcgctcatg	
iroN R	tgtcggtacaggcggttcgtc	iroN, 814
iroN F	ctctggtggtggaagccacc	
fyuA F	acggctttatcctctggccttgg	fyuA, 877
fyuA R	tgaaaacccagtcatcggtgg	
kpsII F	gcgcatttgctgatactgttg	kpsMTII, 581
kpsII R	aggtagttcagactcacacct	
kpsIII F	tcctcttgctactattccccct	kpsMIII, 390
kpsIII R	aggcgtatccatccctcctaac	
chuA.1b	ATGGTACCGGACGAACCAAC	chuA, 288
chuA.2	TGCCGCCAGTACCAAAGACA	
AceK.f	AACGCTATTCGCCAGCTTGC	arpA, 400
ArpA1.r	TCTCCCCATACCGTACGCTA	
yjaA.1b	CAAACGTGAAGTGTCAGGAG	yjaA, 211
yjaA.2b	AATGCGTTCCTCAACCTGTG	
TspE4C2.1b	CACTATTCGTAAGGTCATCC	TspE4.C2, 152
TspE4C2.2b	AGTTTATCGCTGCGGGTCGC	
ArpAgpE.f	GATTCCATCTTGTCAAAATATGCC	Group E arpA, 301
ArpAgpE.r	GAAAAGAAAAAGAATTCCCAAGAG	
trpAgpC.1	AGTTTTATGCCCAGTGCGAG	Group C trpA, 219
trpAgpC.2	TCTGCGCCGGTCACGCCC	

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(1) Clermont O, Christenson JK, Denamur E, Gordon DM. 2013. The Clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 5:58-65.