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MOLECULAR CHARACTERIZATION OF AVIAN PATHOGENIC *ESCHERICHIA COLI* ASSOCIATED WITH EGG PERITONITIS IN COMMERCIAL LAYER CHICKENS

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ABSTRACT

Escherichia coli is a bacterium commonly found as part of the intestinal microflora of many animals. These commensal strains of *E. coli* are harmless and part of the natural environment of the gut. Other more virulent strains of *E. coli* exist; however, with various pathologies in many species. In the poultry industry, one type of *E. coli* is of particular interest; avian pathogenic *E. coli* (APEC). APEC causes extraintestinal infections in poultry including airsacculitis, septicemia, cellulitis, ompholitis/yolk sac infection in young birds, and peritonitis and salpingitis (when combined referred to as salpingoperitonitis) in laying birds. These combined pathologies are known as colibacillosis. Of all these conditions, salpingoperitonitis particularly afflicts the commercial egg laying industry through death and decreased production in layer hens. Furthermore, only few studies have been done to characterize the subpathotype of APEC associated with layer peritonitis. Thus, it is important to add to the knowledge base regarding these pathogenic strains to improve strategies to eliminate the disease. The objective of this study was to characterize a collection of APEC (n=40) isolated from the ovaries, oviducts, and peritoneum of commercial layer hens diagnosed with *E. coli* salpingoperitonitis in comparison to a collection (n=10) of commensal *E. coli* isolated from the ceca of apparently healthy hens. Methods of characterization included virulence genotyping using PCR, phylogenetic grouping, and pulsed-field gel electrophoresis. The results of the study revealed a diverse group of isolates, with a few clusters of apparently identical isolates. Pathogenic isolates were more virulent than commensal isolates, as expected. Atypical results regarding phylogenetic grouping reinforce the need to continue studying different outbreaks of APEC in laying hens.

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Chapter 1

Introduction

Escherichia coli

Escherichia coli is a Gram-negative, facultatively anaerobic bacterium commonly found in the intestines of people and animals. Harmless forms of *E. coli* that inhabit the gut of vertebrates are termed commensal *E. coli*. Commensal *E. coli* are an important component of the microbial flora; it is the predominant aerobic organism in the intestinal tract (14). The bacteria reside in the large intestine, living in and surviving off of nutrients from the mucus layer covering the epithelium. *E. coli* is shed into the intestinal lumen along with degraded mucus and excreted in feces, making the bacteria a common marker used to test for fecal contamination of water and sediment (14). Commensal *E. coli* survive off of nutrients provided by the host and provide some benefit by preventing colonization by pathogens through production of bacteriocins, among other mechanisms (14).

Though some forms of *E. coli* are harmless as described, there are several types of *E. coli* that are pathogenic, causing a variety of infections. These groups are characterized by shared antigens that contribute to the pathogenicity. The O antigens of lipopolysaccharides (LPS) define serogroups while O antigens together with flagellar (H) and/or capsular (K) antigens define serotypes (15). Pathogenic *E. coli* types differ from commensal *E. coli* due to the presence of virulence factors such as adhesins, iron acquisition systems, invasins and toxins which might have acquired from plasmids and bacteriopahges and mobile genetic elements through horizontal gene transfer (15).

Diarrheagenic *E. coli*

The most familiar clinical syndrome caused by pathogenic *E. coli* is enteric/diarrheal disease. There are six established categories of *E. coli* that cause gastrointestinal disease: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli*

(ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC)(15). The EPEC was the first pathotype of *E. coli* to be described after causing large outbreaks of infant diarrhea in the 1940s (15). Since then, EPEC has ceased to be a problem in developed countries but remains a prevalent cause of infant diarrhea in underdeveloped countries. A defining characteristic of EPEC is the attaching and effacing (A/E) method of infecting the intestinal epithelium. The bacteria attach to intestinal epithelial cells and cause cytoskeletal changes. Then, the intestinal microvilli are effaced, forming structures on which the bacteria perch (15). The cause of diarrhea with EPEC infection results from multiple mechanisms including active ion secretion, increased intestinal permeability, intestinal inflammation, and loss of absorptive surface area due to the effacement of microvilli. The EHEC can cause hemorrhagic colitis, non-bloody diarrhea, and hemolytic uremic syndrome. Enterohemorrhagic *E. coli* are easily spread because of their low infectious dose, estimated to be less than 100 cells (15). A characteristic virulence factor for EHEC is Shiga toxin (Stx), also known as verocytotoxin (15). This toxin is produced in the colon and travels by the bloodstream to the kidney, damaging endothelial cells and microvasculature through direct toxicity and recruitment of local cytokine and chemokine production (15). Another pathotype of intestinal pathogenic *E. coli,* ETEC causes a watery diarrhea that varies in severity from self-limiting disease to a severe purging disease (15). The ETEC is especially problematic for children and travelers in developing countries (15). As its name suggests, ETEC uses enterotoxins that induce intestinal secretion. These enterotoxins can be either heat-labile or heat-stable, with different bacteria having potential to release one or the other or both types (15). Enteroaggregative *E. coli* causes persistent diarrhea in children and adults in both developed and developing countries (15). These *E. coli* do not secrete heat-labile or heat-stable enterotoxin and adhere to HEp-2 cells (human cervical adenocarcinoma cell line) in an auto-aggregative pattern (15). All *E. coli* fitting within EAEC may not be pathogenic, but at least a subset has been proven to be human pathogens (15). Another type of intestinal pathogenic *E. coli*, EIEC are closely related to *Shigella* species, sharing essential virulence factors (15). These *E. coli* cause watery diarrhea usually indistinguishable from diarrhea caused by other *E. coli* pathogens (15). The EIEC and *Shigella* have a plasmid-borne type III secretion system that is thought the be the source of most of their pathogenicity, secreting proteins that mediate epithelial signaling events, cytoskeletal rearrangements, cellular uptake, lysis of the endocytic vacuole, and other actions (15). Diffusely adherent *E. coli* have a specific pattern of adherence to HEp-2 cell monolayers and have been implicated as a cause of diarrhea in several studies (15). Diarrheagenic *E. coli* infections are

spread through ingestion of human or animal feces (15). Common sources include contaminated food, raw milk, unpasteurized apple cider, soft cheese, and contaminated water as well as contact with cattle or feces of infected people (16).

Extraintestinal Pathogenic *E. coli*

While gastrointestinal diseases are the most common form of *E. coli* disease, some *E. coli* have the ability to cause extra-intestinal infections (15). These *E. coli* are called extraintestinal pathogenic *E. coli* (ExPEC) and cause various disease in humans and animals. One such type is uropathogenic *E. coli* (UPEC), which are the most common source of bacterial infection in the urinary tract. Like diarrheagenic *E. coli*, UPEC are distinct from commensal *E. coli* (15). Just six serogroups cause about 75% of UTIs, having phenotypes associated with cystitis and acute pyelonephritis in the urinary tract (15). Characteristics of UPEC include expression of P-fimbriae, hemolysin, aerobactin, serum resistance and encapsulation. Instead of ingestion, source of urinary tract infection is most likely UPEC that colonize the colon (15). These bacteria can then infect the periurethral area and ascend the urethra to the bladder (15). Once in the bladder, the change in environment leads to expression of type I fimbriae which attach to transitional epithelial cells triggering apoptosis and exfoliation. In cystitis-causing infections, type I fimbriae continue to be expressed and the infection is limited to the bladder. In other strains causing pyelonephritis, type I fimbriae are not as well expressed, and bacteria instead ascend to the kidneys (15). There, hemolysin can be used to damage the renal epithelium and other products such as lipopolysaccharide can cause an inflammatory response in the host (15). Some strains can penetrate the endothelium between renal tissue and the vasculature, leading to bacteremia (15).

E. coli can also cause neonatal meningitis, these strains being termed NMEC. Neonatal meningitis is a serious affliction causing death in 15-40% of cases and severe neurological defects in many survivors (15). NMEC strains come from a limited number of serogroups and 80% of strains are K1 capsule type (15). The K1 capsule protects the bacteria from the host innate immune system and contains sialic acid which contributes to crossing of the blood-brain barrier (15). Bacteria move from the bloodstream to the central nervous system most likely through a transcytosis process without significant disruption to the blood-brain barrier (15). In bacterial meningitis, the level of bacteremia correlates with the development of meningitis.

Avian Pathogenic *E. coli*

Another type of *E. coli,* and the focus of this thesis, is avian pathogenic *E. coli* (APEC). These strains of *E. coli* cause a wide variety of extra-intestinal infection in many species of birds including turkeys and chickens. The most common types of extra-intestinal infection caused by APEC in birds include airsacculitis, polyserositis, septicemia, and peritonitis (17). These syndromes together are termed colibacillosis. The majority of APEC belongs to O1, O2, and O78 serogroups (17). Colibacillosis has high associated morbidity and mortality rates, making APEC a significant concern in the poultry industry. Birds raised for meat, and eggs are all affected colibacillosis causing multimillion dollar losses in the industry as a whole each year (18). Additionally, it is suggested that there may be a link between APEC and human disease, increasing the interest in controlling and preventing outbreaks of colibacillosis in birds (18). In layer hens in particular, APEC causes a syndrome with acute mortality without prior clinical signs of the disease (19). This form of APEC disease is termed *E. coli* egg peritonitis and it will be the focus of this thesis. Egg peritonitis is one of the most common causes of mortality in laying hens, caused when *E. coli* ascends the oviduct from the cloaca or descends through the respiratory tract (20). Peritonitis borne of respiratory infection is more common in younger birds (20). During egg production, the sphincter between the vagina and the cloaca relaxes due to estrogenic activity. This relaxation can predispose layer hens to egg peritonitis during periods of heavy egg laying (20). Mucosal viral infection is another possible predisposing factor to the development of egg peritonitis (20). As egg peritonitis develops, the oviduct wall becomes thin, distended, and produces a yellow exudate that may expand into the body cavity, sometimes resulting in carcass condemnation (20). Peritonitis specifically occurs once the infection spreads though the oviduct to the peritoneum (20).

Potential virulence factors for APEC include adhesins, iron acquisition systems, serum resistance factors, and toxins (19). While other types of APEC have been well characterized in the literature, knowledge regarding the pathogenesis and detailing the subpathotype of APEC causing layer egg peritonitis is incomplete. In this study, a collection of APEC isolates taken from layer hens with lesions on the peritoneum, ovaries, and oviducts and died of salpingoperitonitis were analyzed. A collection of commensal *E. coli* collected from the ceca of apparently healthy layer hens was included for comparison purposes. Isolates were analyzed for virulence factors using polymerase chain reaction (PCR), phylogenetic typing was conducted to place isolates in one of four groups (A, B1, B2, or D), and pulsed-field gel electrophoresis

(PFGE) was performed to analyze relatedness between the isolates. The objective of this study is to identify the prevalent virulence factors and clonal similarities within our select group of isolates. The results of this study will add to the knowledge base regarding APEC causing salpingoperitonitis in layer hens and later contribute to development of preventative and treatment strategies to prevent production loss.

Chapter 2
Materials and Methods

Bacterial Strains and Media

A collection of APEC isolates (n=40) from the ovaries, oviducts and peritoneum of commercial laying hens diagnosed with *E. coli* salpingitis/peritonitis were used in the study. The isolates originated from the Animal Diagnostic Laboratory at the Pennsylvania State University (University Park, PA) or from commercial layer farms in Pennsylvania. Further information regarding the origin of isolates can be found in Appendix 1. Isolates were obtained by swabbing the lesions at time of necroscopy, followed by plating and incubating aerobically on MacConkey agar at 37°C overnight (Difco, Sparks, MD, USA). Dark pink colonies (lactose fermentation) assumed to be *E. coli* were further confirmed as *E. coli* by API 20E strip test (BioMérieux, France) or Sensitire® Gram-negative identification system (Trek Diagnostic Systems, Cleveland OH). Commensal *E. coli* isolated from ceca or fresh fecal droppings (n=10) were included for comparison purposes. Selected isolates for the study were plated from frozen stocks onto Luria Bertani (LB agar; Difco).

DNA Extraction

The DNA from *E. coli* was extracted by a rapid boiling procedure as described previously (2). Briefly, an isolated colony of *E. coli* grown on MacConkey agar was added to 1 ml of sterile double distilled water in a microcentrifuge tube, and centrifuged at 5000xg for 10 min. The supernatant was removed and 200 μl was added to the tube followed by boiling at 100°C for 10 minutes. The bacterial suspension was then centrifuged at 10,000xg for 10 min. to collect the supernatant containing crude DNA. The extracted DNA samples were stored at -20C° until use.

Virulence Genotyping

Bacterial DNA was amplified using PCR to screen for presence of virulence genes listed in Table 1.

Table 1: Selected Primers

Gene	Primer Forward Sequence	Primer Reverse Sequence	Product Size (bp)	Annealing temperature	Function	Reference
afa8D	GGAAGGAGTGGCGAGTTA	TATATTCATCAGGCCCAA	750	51°C	afimbrial adhesin	3
aufA	ATGGGTCGTAGCATTTCCTG	TTGTGCCTTCCAACCTTTTC	350	55°C	Auf fimbriae	used in this study
cdt-s	GAAAGTAAATGGAATATAAATGTCCG	GAAAATAAATGGAACACACATGTCCG	466	54°C	cytolethal distending toxin	7
fimA MT78	TCTGGCTGATACTACACC	ACTTTAGGATGAGTACTG	266	50°C	main fimbrial unit (variation)	12
FimH	GCAGTCACCTGCCCTCCGGTA	TGAGAACGGATAAGCCGTGG	508	56°C	type I fimbriae	4
ibeA	AAGCAGGGCAATAATTTACTCG	TTTTGCTGTAAGCGTTCCTGT	171	55°C	invasin of brain endothelium A	5
ireA	CCGTGATGTGTTCTGCAGTTAT	AGAGAATCCGTTGCTGCATATT	~1000	56°C	iron acquisition	8
iss	CAGCAACCCGAACCACTTGAT	AGCATTGCCAGAGCGGCAGAA	323	62°C	increased serum survival	6
stgC	TCTGGTTCACATACACTACG	CCAATCATAATCTGGCTTCT	~1000	51°C	Stg fimbriae	9
tia	CCCTTCTGCATCTTGTAAGACA	TATAGGGGCGGTGATAAAAACG	~1000	55°C	invasin/adhesin	10
tsh	GGTGGTGCACTGGAGTGG	AGTCCAGCGTGATAGTGG	~1000	57°C	hemagglutinin autotransporter	11
vat	TCCTGGGACATAATGGTCAG	GTGTCAGAACGGAATTGT	900	55°C	vacuolating cytotoxin	used in this study
chuA	GACGAACCAACGGTCAGGAT	TGCCGCCAGTACCAAAGACA	279	N/A	phylogrouping	1
yjaA	TGAAGTGTCAGGAGACGCTG	ATGGAGAATGCGTTCCTCAAC	211	N/A	phylogrouping	1
TSPE4.C2	GAGTAATGTCGGGGCATTCA	CGCGCCAACAAAGTATTACG	152	N/A	phylogrouping	1

Final concentrations for PCR master mix can be found in Table 2.

Table 2: Polymerase chain reaction components

Component	Concentration	Volume per 50 µl Reaction
10X Buffer (GBiosciences, St. Louis, MO)	1.5 mmol	5 µl
Deoxynucleotide triphosphates (dNTP; GBioSciences)	0.2 mmol	1 µl
Taq polymerase (OMEGA Bio-Tek, Norcross, GA)	0.025 U/µl	0.25 µl
Primers (forward and reverse) (IDT Technologies, Coralville, Iowa)	0.2 mmol	0.5 µl
Double distilled H_2O	-	34.75 µl

PCR cycling conditions were as follows:

1. 94°C for 5 min
2. 94°C for 30 sec
3. Annealing temperature* for 1min
4. 72°C for extension time**
5. Cycle steps 2-4 30x
6. 72°C for 10min
7. 4°C ∞

*Annealing temperatures for each primer can be found in Table 1

**Extension times were adjusted by product size, 30 sec per 500 bp

Phylogenetic Grouping

Phylogenetic grouping was performed as described by Clermont, et al. (1). Isolates were assigned to one of four phylogenetic groups (A, B1, B2, or D) based on the presence of two genes (*chuA* and *yjaA*) and a DNA fragment (TSPE4.C2) using PCR. Further information on the primers used can be found in Table 1. Isolates were assigned according to the decision tree shown in Figure 1. Figure 2 shows an example of a gel used to perform phylogenetic grouping. Following the decision tree provided, lanes 1 and 2 in Figure 2 would be assigned to group A; lane 3 would be assigned to B1, lanes 4 and 5 would be assigned to D, and lanes 6 and 7 would be assigned to B2.

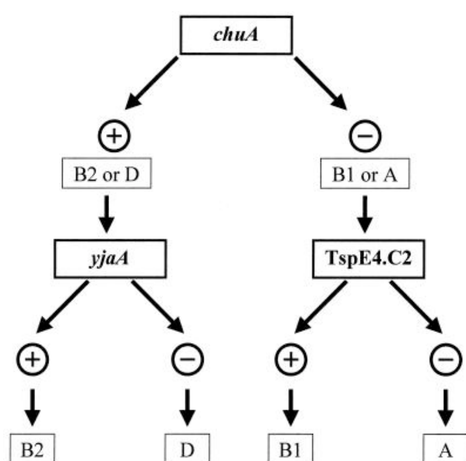

Figure 1: Decision tree for phylogenetic group assignment (1)

Figure 2: Example of gel used for phylogenetic grouping (1)

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis was performed according to PulseNet protocol (13). Briefly, *Xba*I restriction enzyme (Promega Corporation, Madison, WI) digestion was conducted on chromosomal DNA, followed by 24 hours of gel electrophoresis. The CHEF DRII system (Bio-Rad, Marnes-la-Coquette, France) was used for electrophoresis with conditions of an initial time of 2.2 sec, a final time of 54.2 sec at a gradient of 6 V cm-1 and an included angel of 120°. *Salmonella enerica* serotype Braenderup strain H9812 (ATCC® BAA664™, Manassas, VA) was used for standard normalization of gels. BioNumerics 4.0 software (Applied Maths Inc., Austin, Texas) was used to construct a dendrogram using the Dice similarity coefficient and the unweighted-pair group method by average linkages (UPGMA) or neighbor joining algorithm with 3% position tolerance.

Chapter 3

Results

Virulence Genotyping

Figure 3 is a heat map showing the distribution of virulence genes, clustered by similarities between the isolates.

A presence of a gene (positive result) is indicated in red while an absence of a gene (negative result) is indicated in red. Forty APEC isolates studied were grouped into two main clusters based on virulence gene typing (Figure 3). Each main cluster was further divided into subclusters. The most predominant cluster contained isolates 11 isolates, which included APEC isolate numbers 4, 6, 9, 11, 12, 13, 24, 25, 29, 30, and 37. Isolates 4, 6, 9, 11, 12, and 13 originated from the same farm during a single outbreak, and these results suggest one single APEC might have responsible for the outbreak. The most commonly present virulence gene was *fimH*, which encodes type I fimbriae. Other prominent genes included *aufA*, which codes for Auf fimbriae, and *iss*, which codes for a protein associated with increased serum survival.

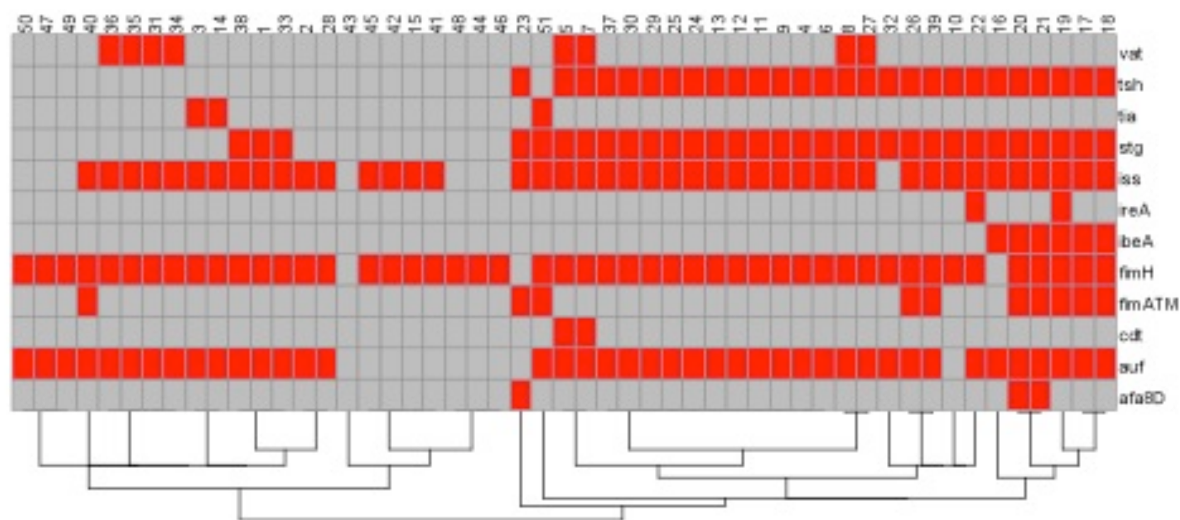

Figure 2: Clustered heat map for virulence genes. Red represents a positive result; grey represents a negative result.

Comparing the occurrence of each gene between APEC and commensal isolates (Table 3), it is evident that certain virulence genes are more common in the pathogenic *E. coli*. For example, *aufA, stgC,* and *tsh* were present in more than half of the APEC isolates, but had little to no representation in commensal isolates. *FimH,* again, was the most commonly present gene, appearing in nearly every APEC and commensal isolate.

Table 3: Virulence gene distribution among APEC and commensal isolates

Gene	**Number of APEC isolates**	**Percentage of APEC isolates**	**Number of commensal isolates**	**Percentage of commensal isolates**
afa8D	3	7.5%	0	0%
aufA	37	92.5%	3	30%
cdt-s	2	5%	0	0%
fimA MT78	8	20%	1	10%
FimH	38	95%	9	90%
ibeA	6	15%	0	0%
ireA	2	5%	0	0%
iss	39	97.5%	4	40%
stgC	31	77.5%	0	0%
tia	2	5%	0	0%
tsh	27	67.5%	0	0%
vat	8	20%	0	0%

Phylogenetic Grouping

Results of phylogenetic grouping for individual can be found in Appendix 1. Table 4 represents the distribution of APEC and commensal isolates into each of the four phylogroups.

Table 4: Distribution of APEC and commensal isolates into 4 phylogroups

Phylogroup	Number of APEC isolates	Percentage of APEC isolates	Number of commensal isolates	Percentage of commensal isolates
A	10	25%	0	0%
B1	1	2.5%	8	80%
B2	0	0%	0	0%
D	29	72.5%	2	20%

Pulsed-Field Gel Electrophoresis

Figure 4 is a clustered dendrogram including percentage similarity.

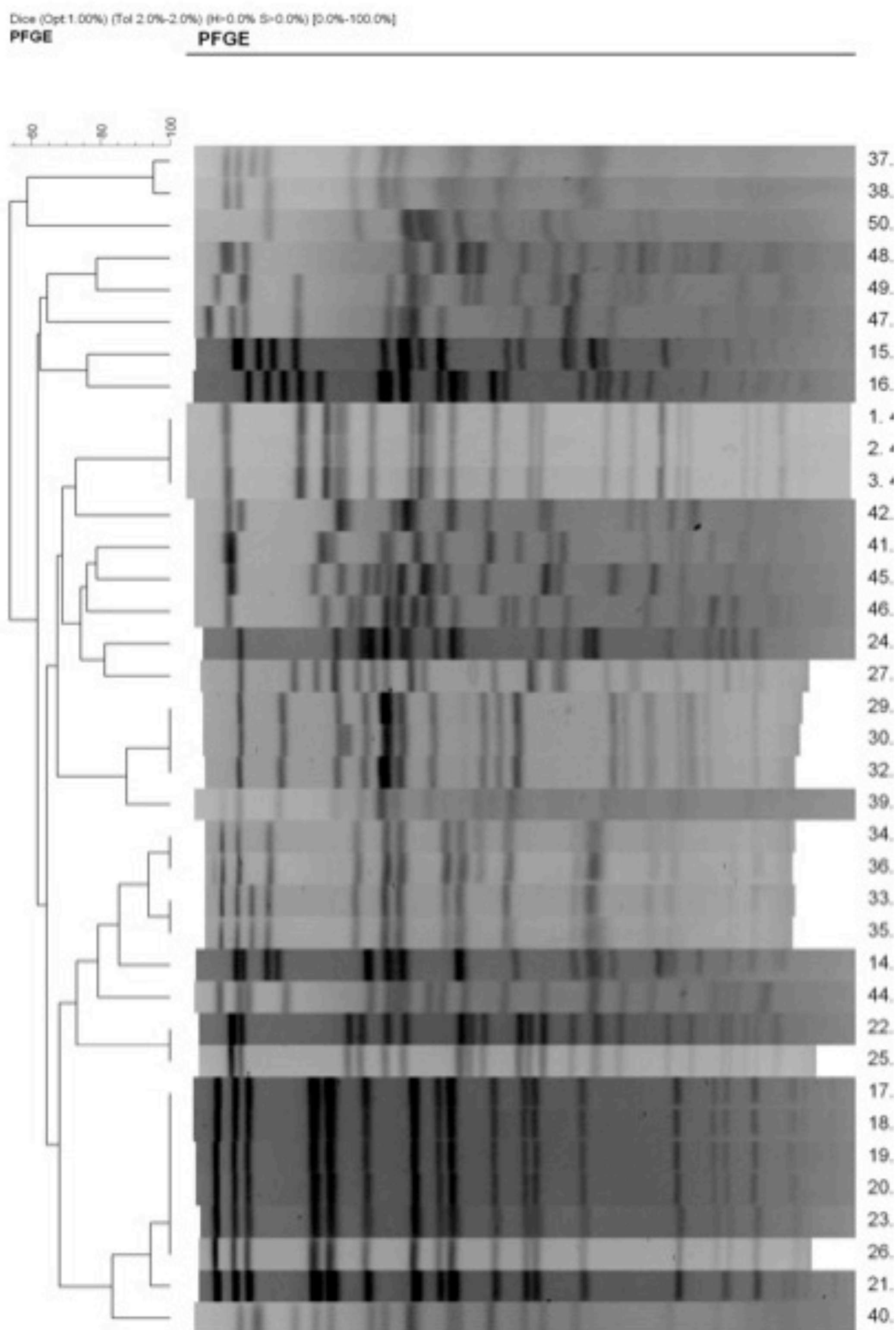

Figure 3: Clustered dendrogram of 37 typeable isolates

Out of 50 total isolates, 37 were typed by *Xba*I digestion where as the other 13 isolates were untypeable with PFGE. Overall, the isolate collection was quite diverse. Typeable isolates showed two major clusters at 60% similarity. These two clusters were then split into 3 and 22 individual clusters, respectively. An important cluster shown above includes isolates 17, 18, 19, 20, 23, and 26. These isolates have identical band patterns and were collected from the same farm, indicating they might be from the same outbreak. Another similar cluster included isolates 1, 2, and 3. Isolates 29, 30, and 32 comprised another small cluster. None of the commensal isolates showed significant relationship to the APEC isolates and none had more than 80% similarity to one another.

Chapter 4

Discussion

All three typing methods used in the present study revealed that APEC are genetically diverse**.** This finding is in consistent with previous studies that used various molecular typing methods to characterize APEC (18). Isolates that clustered together in the PFGE dendrogram were expected to be clustered together in the heat map for virulence genes as well. However, this was not always the case with our results. For example, none of the isolates in the most prominent cluster in the heat map that came from the same farm during a single outbreak (4, 6, 9, 11, 12, and 13) were typeable for PFGE. While this does not discount the likelihood that a single APEC caused these birds to die in this particular outbreak, it could not be confirmed by PFGE. Furthermore, not every isolate collected at this specific outbreak is contained within the heat map cluster, indicating genetic differences between bacteria infecting some of the affected birds. Isolates 5 and 7 possess two additional genes while isolate 10 is missing one. These differences could be due to different plasmid profiling between closely related strains of APEC as a result of horizontal gene transfer.

The most prominent cluster in the PFGE dendrogram, including isolates 17, 18, 19, 20, 23, and 26 do not share all the same virulence genes based on the heat map. Because PFGE is dependent on enzyme restriction, this discrepancy may be due to different genes or regions within fragments of the same size between isolates. This trend is followed with most of the results. Despite the isolates clustering together with PFGE, the virulence profiles between isolates were different.

In terms of phylogeny most ExPEC in the literature fall into groups D and B2 (21). Commensal strains generally fall in groups A and B1 (21). Our results did not all fall within these parameters. While the majority of APEC isolates (75%) fell in groups D or B1, a significant portion (25%) were in group A. We had inconsistent results within the commensal isolates as well; 80% were group B1, as expected, but 20% fell into group D. However, the virulence gene profiles of the commensal isolates that belonged to phylogenetic group indicated that they are indeed commensal isolates. For example, two isolates that fell into group D (43 and 46) only isolate 46 possessed a virulence gene. That gene was *FimH,* which was the most widely

represented gene between all isolates including the commensal *E. coli*. In consistence with the previous studies (22) *fimH* was absent only from 2 total isolates examined in the present study. Generally, as described in the literature, APEC isolates carried more virulence genes than commensal isolates.

The results of the study overall yielded results consistent with the literature for differences between pathogenic and commensal isolates, but some inconsistent results within the pathogenic isolate pool, especially regarding phylogeny. These results reinforce the need for further study on APEC, focusing on molecular characterization.

Appendix A

Origin and Phylogenetic Groups of Isolates

	Isolate ID	Isolate number	Origin	Phylogenetic group
Peritonitis	4.0022	1	Ovary, commercial layer with peritonitis	D
	4.0023	2	Ovary, commercial layer with peritonitis	D
	4.0024	3	Peritoneum, commercial layer with peritonitis	D
	7.1197	4	Ovary, commercial layer with peritonitis	A
	7.1198	5	Ovary, commercial layer with peritonitis	A
	7.1199	6	Ovary, commercial layer with peritonitis	A
	7.1200	7	Ovary, commercial layer with peritonitis	A
	7.1201	8	Ovary, commercial layer with peritonitis	A
	7.1202	9	Ovary, commercial layer with peritonitis	A
	7.1203	10	Ovary, commercial layer with peritonitis	A
	7.1204	11	Ovary, commercial layer with peritonitis	A
	7.1205	12	Peritoneum, commercial layer with peritonitis	A
	7.1206	13	Ovary, commercial layer with peritonitis	A
	00196 ovary	14	Ovary, commercial layer with peritonitis	D
	078 ovary 00964	15	Ovary, commercial layer with peritonitis	D
	P1018870	16	Ovary, commercial layer with peritonitis	D
	1/22-1 OD	17	Oviduct, commercial layer with peritonitis	D
	1/22-2 OD	18	Oviduct, commercial layer with peritonitis	D
	1/22-4 OD	19	Oviduct, commercial layer with peritonitis	D
	1/22-7 OD	20	Oviduct, commercial layer with peritonitis	D
	1/22-7-2 OD	21	Oviduct, commercial layer with peritonitis	D
	1/22-8 OD	22	Oviduct, commercial layer with peritonitis	D

	1/22-9 OD	23	Oviduct, commercial layer with peritonitis	D
	1/22-10 OD	24	Oviduct, commercial layer with peritonitis	B1
	1/22-11 OD	25	Oviduct, commercial layer with peritonitis	D
	1/22-12 OD	26	Oviduct, commercial layer with peritonitis	D
	3/22 perit 7 OD	27	Oviduct, commercial layer with peritonitis	D
	3/22 perit 3 OD	28	Oviduct, commercial layer with peritonitis	D
	3/22 perit 2 OD	29	Oviduct, commercial layer with peritonitis	D
	3/22 perit 6 OD	30	Oviduct, commercial layer with peritonitis	D
	3/22 perit 4 OD	31	Oviduct, commercial layer with peritonitis	D
	3/22 perit 1 OD	32	Oviduct, commercial layer with peritonitis	D
	4/3 perit 7 OD	33	Oviduct, commercial layer with peritonitis	D
	4/3 perit 5 OD	34	Oviduct, commercial layer with peritonitis	D
	4/3 perit 6 OD	35	Oviduct, commercial layer with peritonitis	D
	4/3 perit 4 OD	36	Oviduct, commercial layer with peritonitis	D
	4/3 perit 1 OD	37	Oviduct, commercial layer with peritonitis	D
	4/3 perit 2 OD	38	Oviduct, commercial layer with peritonitis	D
	5/10 perit 3 OD	39	Oviduct, commercial layer with peritonitis	D
	5/10 perit 4 OV	40	Ovary, commercial layer with peritonitis	D
Commensals	1-1SI	41	Cecal contents, healthy commercial layer	B1
	2-1LI	42	Cecal contents, healthy commercial layer	B1
	3-1caeca	43	Cecal contents, healthy commercial layer	D
	4-2SI	44	Cecal contents, healthy commercial layer	B1
	5-2LI	45	Cecal contents, healthy commercial layer	B1
	6-2caeca	46	Cecal contents, healthy commercial layer	D
	7-3LI	47	Cecal contents, healthy commercial layer	B1
	8-3caeca	48	Cecal contents, healthy commercial layer	B1
	9-4SI	49	Cecal contents, healthy commercial layer	B1
	10-4LI	50	Cecal contents, healthy commercial layer	B1

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ACADEMIC VITA

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EDUCATION	**The Pennsylvania State University- Schreyer Honors College,** University Park, PA	May 2014—Projected
	B.S. in Veterinary and Biomedical Sciences	
RESEARCH	**Cell Biology and Neuroscience,** Rutgers University	Summer 2013
	NeuroSURP Research Intern	
	▪ *Contributed to poster for 2013 New Jersey Stem Cell Symposium* ▪ *Employed CRISPR/Cas genome editing technology to modify ATM gene in HEK293 and induced pluripotent stem cells* ▪ *Skills developed include iPSC and HEK cell culture techniques, RT-qPCR*	
	Microbiology Section of the Animal Diagnostic Laboratory, Penn State University	Fall 2011-Present
	Independent Research	
	▪ *Several projects studying genetic makeup of E. coli and salmonella isolates* ▪ *Skills developed include PCR, gel electrophoresis, plasmid extraction, plate streaking, bacteria inoculation in broth, execution of boiled preps, and preparation of frozen stocks* ▪ *Currently pursuing thesis research on gene expression in avian* E. coli *isolates*	
PROFESSIONAL EXPERIENCE	**Ortho Clinical Diagnostics,** Raritan, New Jersey	Summer 2012
	Intern	
	▪ *Performed hemolysis and red blood cell concentration testing in the Blood Bank Quality Analysis Lab* ▪ *Entered hemolysis and red blood cell concentration data for tracking and trending* ▪ *Assisted in laboratory start-up*	

	Private Elementary/Intermediate School Tutor ▪ *Tutored students entering grades 3 and 4 in Math and English in a one-on-one home setting* ▪ *Developed lesson plans for 90-minute sessions*	Summer 2011
	Kumon Learning Center, Bridgewater, New Jersey Tutor ▪ *Tutored students grades K-12 in Math and English* ▪ *Graded students' work and provided guidance in learning new topics* ▪ *Instructed one-on-one reading lessons with studen*	Summer 2010
ACTIVITIES	**H.E.A.L. (Help Every Angel Live)** Canning Executive Chair for Penn State THON Special Interest Group ▪ *Organize and participate in canning and canvassing fundraising trips raising ~$12,000 per weekend for the Penn State Dance Marathon* ▪ *Lead organization of ~60 active members along with other members of the executive board*	April 2012- Present
	Schreyer Honors College Day of Service Marketing Chair ▪ *Responsible for advertising and merchandise for the day of service* ▪ *Facilitated the daylong event, which brought in ~50 volunteers*	Fall/Spring 2011-2012
HONORS	**Dean's List**—Semester GPA greater than or equal to 3.50	Fall 2010-2012, Spring 2011, Spring 2013